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Review

Metabolism of cyanogenic glycosides: A review

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ABSTRACT

Potential toxicity of cyanogenic glycosides arises from enzymatic degradation to produce hydrogen cyanide. Information on the metabolism of cyanogenic glycosides is available from *in vitro*, animal and human studies. In the absence of β-glucosidase enzymes from the source plant material, two processes appear to contribute to the production of cyanide from cyanogenic glycosides; the proportion of the glycoside dose that reaches the large intestine, where most of the bacterial hydrolysis occurs, and the rate of hydrolysis of cyanogenic glycosides to cyanohydrin and cyanide. Some cyanogenic glycosides, such as prunasin, are actively absorbed in the jejunum by utilising the epithelial sodium-dependent monosaccharide transporter (SGLT1). The rate of cyanide production from cyanogenic glycosides due to bacterial β-glycosidase activity depends on; the sugar moiety in the molecule and the stability of the intermediate cyanohydrin following hydrolysis by bacterial β-glucosidase. Cyanogenic glycosides with a gentiobiose sugar, amygdalin, linustatin, and neolinustatin, undergo a two stage hydrolysis, with gentiobiose initially being hydrolysed to glucose to form prunasin, linamarin and lotaustralin, respectively. While the overall impact of these metabolic factors is difficult to predict, the toxicity of cyanogenic glycosides will be less than the toxicity suggested by their theoretical hydrocyanic acid equivalents.

1. Introduction

A number of plants and associated plant-based foods naturally contain cyanogenic glycosides. There are approximately 25 cyanogenic glycosides known. The major cyanogenic glycosides found in the edible parts of plants are amygdalin (almonds, stone fruit, pome fruit), dhurrin (sorghum), linamarin (cassava, lima beans, linseed/flaxseed, spinach), linustatin (cassava, linseed/flaxseed), lotaustralin (cassava, lima beans), prunasin (stone fruit, pome fruit, pip fruit), and taxiphyllin (bamboo shoots) (Codex Committee on Contaminants in Foods, 2008; Haque and Bradbury, 2002).

The basic structure of cyanogenic glycosides includes a core carbon attached to a −CN moiety and two substituent groups (R1 and R2) and attached to a sugar, either a monosaccharide (glucose) or a disaccharide (gentiobiose) by a glycosidic bond.

R1 may be a methyl group or a phenyl or *p*-hydroxyphenyl group. R2 is most commonly hydrogen, but may also be a methyl or ethyl group. Table 1 gives the structure details of the major cyanogenic glycosides found in human foods.

It is immediately apparent that there is a high degree of structural relatedness within this group of compounds. Dhurrin and taxiphyllin are stereoisomers (epimers), as are prunasin and sambunigrin. Removal of a single sugar converts amygdalin to prunasin, linustatin to linamarin, and neolinustatin to lotaustralin.

Release of hydrogen cyanide from cyanogenic glycosides can occur through enzymatic hydrolysis by endogenous β-glycosidase, following maceration or wounding of the plant, or by gut microflora, following ingestion of the plant material (Codex Committee on Contaminants in Foods, 2008). The glycoside is enzymatically converted to the corresponding cyanohydrin, which then spontaneously decomposes to form hydrogen cyanide (HCN) and a ketone or aldehyde.

In linseed/flaxseed this process involves two distinct β-glucosidase enzymes (Shahidi and Wanasundara, 1997). Linustatinase is able to hydrolyse the gentiobiose moiety of linustatin and neolinustatin to glucose, producing linamarin and lotaustralin, respectively. A linamarase β-glucosidase then hydrolyses these glycosides to the corresponding cyanohydrin.

Potential toxicity of cyanogenic glycosides arises from enzymatic degradation to produce hydrogen cyanide, resulting in acute cyanide poisoning. Clinical symptoms of acute cyanide poisoning include rapid respiration, drop in blood pressure, rapid pulse, headache, dizziness, vomiting, diarrhea, mental confusion, stupor, blue discolouration of the skin due to lack of oxygen, twitching and convulsions (FSANZ, 2004; Speijers, 1993).

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Table 1
Cyanogenic glycosides occurring in human foods.

Name	R1[a]	R2[a]	Sugar	Foods
Amygdalin	Phenyl	H	Gentiobiose (R)	Stone fruit, pome fruit
Dhurrin	*p*-hydroxyphenyl	H	Glucose (S)	Sorghum
Linamarin	Methyl	Methyl	Glucose	Cassava, lima beans, linseed
Linustatin	Methyl	Methyl	Gentiobiose	Linseed
Lotaustralin	Methyl	Ethyl	Glucose (R)	Cassava, lima beans, linseed
Neolinustatin	Methyl	Ethyl	Gentiobiose (R)	Linseed
Prunasin	Phenyl	H	Glucose (R)	Ferns
Sambunigrin	Phenyl	H	Glucose (S)	Elderberries
Taxiphyllin	*p*-hydroxyphenyl	H	Glucose (R)	Bamboo shoots

[a] Substituent groups attached to the core carbon.

Several diseases are associated with chronic dietary intake of cyanogenic glycosides, although there is some debate over the causal relationships due to confounding nutritional factors (Davis, 1991; FSANZ, 2004; Speijers, 1993). For example, malnourished individuals appear to be more susceptible to the effects of cyanogenic glycosides. Populations with a reliance on cyanogenic foods, such as in African regions where cassava is a staple, have developed food preparation procedures that largely detoxify the food. However, the cyanogenic glycoside content of available food and adherence to detoxifying procedures are reported to be variable. Diseases associated with chronic dietary intake of cyanogenic glycosides include:

- Konzo is a motor neuron disease characterised by irreversible weakness in the legs. In severe cases, patients are not able to walk, and speech and arm movement may be affected. Konzo particularly affects children and women of childbearing age in East Africa in times of food shortage and is associated with a high and sustained intake of cassava (*Manihot esculenta* Crantz) in combination with a low intake of protein (Davis, 1991; FSANZ, 2004).
- Tropical ataxic neuropathy (TAN) describes several neurological symptoms affecting the mouth, eyesight, hearing or gait of mostly older males and females. TAN is attributed to cyanide exposure from the chronic consumption of foods derived from cassava (FSANZ, 2004).
- Goitre and cretinism due to iodine deficiency can be exacerbated by chronic consumption of insufficiently processed cassava. Cyanogenic glycosides from cassava are detoxified to thiocyanate that competes with iodine in the thyroid, effectively increasing the dietary requirement for iodine (FSANZ, 2004).

The current study was initiated to review available information on the kinetics of decomposition of plant cyanogenic glycosides to hydrogen cyanide in the human gut. This is to determine if human gut microflora are able to release cyanide sufficiently quickly from foods containing cyanogenic glycosides to potentially cause toxic effects in humans. This information was considered to be important to refine risk assessments (Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2012).

2. Methods

2.1. Information recovery strategies

Searches of the scientific literature were carried out in Scopus and PubMed. Searches for University theses and dissertations were carried out in Networked Digital Library of Theses and Dissertations (NDLTD) and a selection of university-specific databases.

Searches were also made of specific sites of organisations responsible for food safety assessment European Food Safety Authority (EFSA), Food Standards Australia New Zealand (FSANZ), Food Standards Agency (FSA), International Programme on Chemical Safety (IPCS, INCHEM) and the United States Food and Drug Administration (FDA).

A general search of the Internet was also carried out using the Google search engine.

Search terms used were; cyanogenic (or cyanogen*), cyanide, linamarin, lotaustralin, amygdalin, prunasin, sambunigrin, dhurrin, linustatin, neolinustatin, taxiphyllin, metabolism (or metab*), uptake, absorption, distribution, excretion, kinetics (or kinet*), toxicokinetics, and pharmacokinetics.

For scientific papers that were found to be pertinent, reference lists were checked for further pertinent papers. Subsequent papers citing the reference were also checked for pertinence.

Given the paucity of papers recovered on this topic, no exclusion criteria were applied. Instead, the review sought to identify the strengths and weaknesses of each paper considered. Most papers recovered were from the 1980s and 1990s, with few more recent relevant papers.

In total, 44 documents were recovered and considered to contain information relevant to the metabolism of cyanogenic glycosides. A summary of the type of publication, publication date and cyanogenic glycosides covered is given in Table 2.

3. Results and discussion

3.1. In vitro studies

Table 3 summarises the *in vitro* studies reviewed.

3.1.1. Comparative studies

Crude enzyme preparations were produced from the caecum of female golden Syrian hamsters and mixed with solutions of linamarin, amygdalin or prunasin (Frakes et al., 1986). Liberated cyanide was measured over a 40-min period. Experiments were conducted at a range of cyanogenic glycoside (substrate) concentrations and Lineweaver-Burk plots used to estimate enzyme kinetic parameters.

Cyanide was liberated in a linear manner with time for prunasin and linamarin, but 708 nmol of cyanide were liberated from 10^{-3} M prunasin in 40 min, while only 64 nmol of cyanide were liberated from an equal concentration of linamarin. The plot of cyanide liberation against time for amygdalin was curvilinear, with 294 nmol of cyanide released in 40 min. The authors hypothesised that enzymatic hydrolysis of amygdalin proceeds by a two-step process, with one glucose molecule removed to convert amygdalin to prunasin. The rate of cyanide liberation from amygdalin approached that for prunasin in the latter stages of the hydrolysis.

Cyanogenic glycosides (prunasin, linamarin, amygdalin) were added to bovine ruminal fluid *in vitro* (Majak et al., 1990). The rate of cyanide production was highest for prunasin, followed by linamarin, with amygdalin producing cyanide at the lowest rate. Animal feeding practices influenced cyanide production rates, presumably due to the

Table 2
Summary of publications considered.

Decade of publication	Publication type	Cyanogenic glycosides considered[a]
Pre-1970 (3)	Journal paper (38)	Amygdalin (16)
1970s (3)	Reports (4)	Linamarin (13)
1980s (19)	Book chapters (2)	Prunasin (4)
1990s (9)		Dhurrin (1)
2000s (6)		
2010s (4)		

[a] The total of studies in this category is less than the total number of studies, as some studies used plant material rather than purified glycosides. Also, some studies considered more than one glycoside.

Table 3
Summary of *in vitro* studies reviewed on the metabolism of cyanogenic glycosides.

Compound	Study type	Reference
Limamarin	Cyanide liberation in presence of caecal material	Frakes et al. (1986)
	Cyanide liberation in presence of bovine ruminal fluid	Majak et al. (1990)
	Metabolism by rat liver microsomal preparations	Maduagwu (1989)
	Cyanide liberation by gut bacteria	Fomunyam et al. (1984)
Amygdalin	Cyanide liberation in presence of caecal material	Frakes et al. (1986)
	Cyanide liberation in presence of bovine ruminal fluid	Majak et al. (1990)
	Cyanide liberation by rat gastrointestinal contents	Hill et al. (1980); Newton et al. (1981)
	Cyanide liberation by gut bacteria	Newton et al. (1981)
	β-glucosidase activity in germ-free rats	Newmark et al. (1981)
	Metabolism in simulated human upper gastrointestinal tract fluids	Shim and Kwon (2010)
	Metabolism in rat small intestines	Strugala et al. (1986)
	Intestinal absorption in rat intestines	Wagner and Galey (2003)
Prunasin	Cyanide liberation in presence of caecal material	Frakes et al. (1986)
	Cyanide liberation in presence of bovine ruminal fluid	Majak et al. (1990)
	β-glucosidase activity in germ-free rats	Newmark et al. (1981)
	Intestinal absorption in rat intestinal sections	Strugala et al. (1995)
	Intestinal absorption in rat intestines	Wagner and Galey (2003)

quantity and type of microbes produced. The authors suggested that the rate difference between prunasin and linamarin may have been due to the relative stability of the intermediate cyanohydrins, with benzaldehyde cyanohydrin (from prunasin) dissociating at a much higher rate (k = 0.0354/s at 24 h) than acetone cyanohydrin, from linamarin (k = 0.0069/s at 24 h). The lower rate of cyanide production from amygdalin was ascribed to the extra hydrolytic step required.

3.1.2. Linamarin

Linamarin rapidly disappeared in rat liver microsomal preparations at pH 6.0–6.5, without production of detectable HCN or thiocyanate (Maduagwu, 1989). Boiled microsomal preparations did not degrade linamarin. The authors could not determine whether HCN was produced and then rapidly detoxified by a mechanism that did not result in thiocyanate production or whether the metabolism of linamarin to did not result in HCN production.

It was hypothesised that intestinal hydrolysis of linamarin may be performed by β-glucosidase from enteric flora and it was shown that *Escherichia coli* and *Streptococcus faecalis* were able to liberate hydrogen cyanide from linamarin in culture (Fomunyam et al., 1984). Another study found that cyanide was liberated from linamarin by various strains of *Klebsiella*, but not *E. coli, Clostridium* or *Proteus* (Bourdoux et al., 1980).

3.1.3. Amygdalin

Rat gastrointestinal tracts were serially divided, minced and incubated with amygdalin (Hill et al., 1980). Very little cyanide was released by the contents of the stomach and upper intestine, but activity increased sharply in the lower intestine and faeces.

A similar study with divided rat gastrointestinal tract demonstrated cyanide release by all regions of the gut, but maximal release by the caecum (Newton et al., 1981). Four bacterial species commonly found in human faeces (*Enterobacter aerogenes, Streptococcus fecalis, Clostridium perfringens* and *Bacteroides fragilis*) were shown to be capable of releasing cyanide from amygdalin, with *B. fragilis* by far the most efficient at releasing cyanide. *Escherichia coli* did not liberate cyanide. Cell-free filtrates of bacterial broth cultures did not liberate cyanide. Cyanide was not liberated from amygdalin by acidic (pH 2.2–4.1) human gastric juice, but some cyanide was released as the pH of the gastric juice increased above 5.0, pHs at which bacterial colonisation of the stomach may occur.

Newmark et al. studied β-glucosidase activity of germ-free rat and human intestinal tissue by measuring release of glucose following incubation with amygdalin or prunasin (Newmark et al., 1981). Germ-free rats are specially bred and raised to have no microorganisms in or on them, including no gut microflora. Enzyme activity was detected in germ-free rat kidney, small intestinal mucosa, and small intestinal contents, but not stomach or colonic contents. A range of human normal and neoplastic tissues were taken at autopsy and similarly assessed. Only normal ileal tissue exhibited any detectable β-glucosidase activity against amygdalin and prunasin. This study is somewhat difficult to interpret, as the end point measured is glucose release and not cyanide generation.

Digestion of amygdalin in simulated human upper gastrointestinal tract fluids (salivary/gastric/small intestinal) identified prunasin as the major metabolite (Shim and Kwon, 2010). Application of the digesta to a human intestinal cell culture system (CACO-2) resulted in degradation of prunasin by cellular β-glucosidase enzymes to form the cyanohydrin, which then underwent hydroxylation across the small intestinal wall. Hydroxylation was at the *meta*-position of the phenyl group.

Intestinal first pass metabolism of amygdalin was investigated in rat small intestines *in vitro* (Strugala et al., 1986). Hydrolysis of amygdalin to prunasin was shown to occur in the proximal jejunum, through a specific β(1–6) cleavage of the terminal glucose. Caecal contents of conventional rats exhibited both amygdalin and prunasin hydrolysing activity. No metabolic activity toward amygdalin or prunasin was exhibited by isolated rat livers within 2 h.

3.1.4. Prunasin

Transport of prunasin from the small intestine into the portal circulation (mucosa to serosa) was studied in rat (female Sprague-Dawley) intestinal sections (Strugala et al., 1995). The evidence strongly suggested that absorption in the jejunum (the middle section of the small intestine) was by active transport, utilising the small intestine glucose carrier system. This conclusion was supported by experiments that demonstrated that absorption was saturable, dependent on sodium ion concentration on the mucosal side, and temperature dependent. Absorption in the ileum did not exhibit these characteristics and was assumed to occur by passive diffusion. The authors speculated that other cyanogenic glycosides may be absorbed by this active transport mechanism. The implications of this finding are that certain cyanogenic glycosides may by significantly absorbed in the small intestine, prior to encountering the regions of highest cyanide liberating potential in the distal large intestine.

This absorption mechanism was further investigated, also using everted rat intestines (Wagner and Galey, 2003). The ability of compounds to utilise the epithelial sodium-dependent monosaccharide transporter (SGLT1) was determined by their ability to decrease the rate of transport of radio-labelled glucose and mannose. Prunasin (1 mM) was found to significantly inhibit transport of glucose and mannose, while amygdalin (1 mM and 3 mM) had little or no impact on the rate of transport. This adds weight to the hypothesis that cyanogenic

Table 4
Summary of animal studies reviewed on the metabolism of cyanogenic glycosides.

Compound	Study type	Dose level (mg/kg body weight)	Reference
Linamarin	Blood cyanide after administration, Syrian hamster	109	Frakes et al. (1986)
	Faecal, urinary and blood analysis after administration, rat	300	Barrett et al. (1977); Umoh et al. (1986)
	Faecal, urinary and blood analysis after administration, rat	10–75	Okafor and Maduagwu (1999)
	Faecal, urinary and blood analysis after administration, rat	NS	Jansz and Inoka Uluwaduge (1997)
	Toxicity in methionine deficient or sufficient rats	250 or 500	Philbrick et al. (1981)
	Impact of protein nutrition on toxicity in rats	300	Umoh et al. (1986)
Amygdalin	Blood cyanide after administration, Syrian hamster	201	Frakes et al. (1986)
	Toxicity in germ-free and normal rats	50 or 600	Carter et al. (1980)
	Toxicity and blood cyanide comparison between amygdalin and KCN and between oral and intraperitoneal administration	400-2220	Hill et al. (1980)
	Urinary thiocyanate in normal and antibiotic-treated rats. Comparison of oral and intravenous administration	250	Newton et al. (1981)
	Toxicity and urinary excretion in rats, with and without β-glucosidase	200, 300, 400 or 600	Adewusi and Oke (1985)
	Comparison of urinary excretion between oral and intravenous administration, Beagle dogs and rats	~50 (dog) 50 mg (rat)[a]	Rauws et al. (1982)
	Appearance of metabolite (prunasin) in plasma, rat	300	Chen et al. (2012)
	Urinary analysis for metabolites, rat	100	Ge et al. (2007)
Prunasin	Comparison of metabolism of oral and intravenous administration, dog	10	Rauws et al. (1982–1983)
Prunasin/sambunigrin	Toxicity and urinary excretion	300, 400, 500, 630 or 1000	Sakata et al. (1987)
Dhurrin	Dhurrin and cyanide in intestinal contents, faeces, urine and plasma	NS	Okoh et al. (1988)

NS: not stated.
[a] The body weight of the rats used in this study was not given.

glycosides containing disaccharides do not undergo active transport in the small intestine.

3.2. Animal studies

Table 4 summarises the animal studies reviewed.

3.2.1. Comparative studies

Female golden Syrian hamsters received oral doses of 0.44 mmol/kg body weight of linamarin or amygdalin (Frakes et al., 1986). Blood cyanide levels were higher (approximately double) in linamarin dosed animals than amygdalin dosed animals after 30 min. However, from 1 h after dosing until the end of the study blood cyanide levels were higher in the amygdalin dosed animals. Similar peak blood cyanide concentrations were achieved in the two groups, but the peak occurred after 1 h for the amygdalin group and after 3 h for the linamarin group. Blood cyanide levels for both groups dropped sharply between three and 4 h after dosing, while blood thiocyanate concentrations increased steadily throughout the study period for both groups.

3.2.2. Linamarin

Male Wistar rats ($n = 6$) were administered 30 mg of pure linamarin by stomach tube (Barrett et al., 1977). No linamarin was recovered in the faeces, suggesting either complete absorption of linamarin from the gastrointestinal (GI) tract, metabolism to other compounds in the GI tract or a combination of both. Analysis of urine recovered 5.65 mg (19% of initial dose) of intact linamarin, confirming absorption of intact linamarin. Thiocyanate, the detoxification product of hydrogen cyanide, was also detected in the urine, in amounts equivalent to a further 7.1 mg (24%) of linamarin. Linamarin was not detected in rat blood. The lack of linamarin in faeces and blood was confirmed by a subsequent study (Umoh et al., 1986).

Very similar results were obtained in a study comparing metabolism of pure linamarin or a crude cyanogenic glycoside preparation from cassava in male albino Wistar rats (Okafor and Maduagwu, 1999). No intact linamarin was detected in the blood or faeces and approximately 20% of the ingested dose was recovered intact in a 24-h urine sample, when either linamarin (18.2–24.3%) or the crude cassava preparation (17.6–25.2%) were fed. However, as the administered dose of linamarin was increased from 1.0 to 7.5 mg/100 g body weight, the percentage of the dose recovered intact from the urine decreased. Urinary thiocyanate also increased with increasing linamarin ingestion.

A radiotracer study was carried out, in which linamarin was labelled with ^{14}C at the CN carbon and fed to rats (Jansz and Inoka Uluwaduge, 1997). The study found:

- Intact linamarin appeared in the urine
- No radiocarbon appeared in the faeces
- Radiocarbon-labelled cyanide and thiocyanate were not observed in blood
- An unknown radiocarbon-labelled metabolite appeared first in the portal circulation, then in the peripheral circulation and then in urine
- The bulk of the radiocarbon was not excreted after 6 days

From the results of this radiotracer study, it was hypothesised that either; linamarin was metabolised to an unknown metabolite, that was subsequently catabolised, or linamarin was hydrolysed to cyanide, which was converted to β-cyanoalanine in a fast reaction and then to asparagine.

This and subsequent studies supported the proposition that linamarin could be converted to hydrogen cyanide in the body, without the need for the presence of plant linamarase (Philbrick et al., 1977).

Male Wistar rats ($n = 54$ per dose group) received a single oral dose of either 250 or 500 mg/kg body weight linamarin (Philbrick et al., 1981). All rats receiving 500 mg/kg body weight linamarin died within 5 h, exhibiting symptoms of cyanide intoxication. Rats receiving 250 mg/kg body weight linamarin survived longer, but exhibited toxicity signs. Rats in this group were either on a methionine deficient diet or a diet supplemented with methionine. It has been reported that methionine acts as a sulphur source for detoxification of cyanide to thiocyanate (Barrett et al., 1978). Of those on the methionine deficient diet, 50% died within 9 h, while only 10% of methionine supplemented rats died. Umoh et al. (1986) demonstrated in male Wistar albino rats receiving adequate protein nutrition resulted in linamarin and its detoxification by-products being excreted in the first 24 h after ingestion. For malnourished rats, excretion was delayed and occurred predominantly up to 48 h after ingestion, giving a longer period for toxic

effects to be exerted.

3.2.3. Amygdalin

Rats (Sprague-Dawley) given a single oral dose of amygdalin (600 mg/kg) died, usually within 2 h (Carter et al., 1980). Germ-free rats receiving the same dose of amygdalin showed no visible signs of toxicity. The conventional rats had high blood cyanide levels, while the germ-free rats had blood cyanide levels indistinguishable from rats not dosed with amygdalin. These results were explained in terms of the necessary role of gut microfloral β-glucosidases in the degradation of amygdalin. The experiment was repeated with non-lethal doses (50 mg/kg). Intact amygdalin was recovered to a greater extent in germ-free rats, with amygdalin recovered from both urine and faeces. In conventional rats, amygdalin was only recovered from urine and a lower recovery of amygdalin was observed, presumably due to the proportion of dose that was degraded to HCN.

Female mice (CD2F1) received oral amygdalin (400–2220 mg/kg) or intraperitoneal potassium cyanide (KCN; 3–6 mg/kg) (Hill et al., 1980). For amygdalin-dosed mice, maximum blood cyanide levels were reached 1.5–2 h after dosing, while for KCN maximum blood cyanide levels were reached within 20 min. The LD_{10} for intraperitoneal KCN was approximately 4.2 mg/kg (1.7 mg/kg HCN equivalents), while the LD_{10} for oral amygdalin was approximately 450 mg/kg (26.6 mg/kg HCN equivalents). This suggests that as little as 6% of amygdalin may be hydrolysed to form cyanide. Intraperitoneal injection of amygdalin (5000 mg/kg) produced no signs of toxicity or significant increase in blood cyanide, confirming the importance of enteric microflora for the hydrolysis of amygdalin.

An oral LD_{50} of 522 mg/kg was determined for amygdalin in female Fischer 344 rats (Newton et al., 1981). The first signs of neurological damage were observed approximately 80 min after administration. To study the kinetics of amygdalin, rats were given a sub-lethal oral dose (250 mg/kg), with a proportion of rats being treated with neomycin (a broad spectrum antibiotic) prior to receiving amygdalin. Neomycin-treated rats showed a minimal increase in urinary thiocyanate following amygdalin administration, while non-neomycin-treated animals showed a 40-fold increase in urinary thiocyanate. Non-neomycin-treated rats were compared after administration of oral and intravenous amygdalin. Urine was monitored for thiocyanate and benzaldehyde (as a measure of intact amygdalin). Urinary thiocyanate increased in rats receiving amygdalin intravenously, with the equivalent of 6% of the administered dose recovered. For rats receiving amygdalin orally, approximately twice as much of the administered dose was recovered in the form of thiocyanate. In intravenous studies, 70–85% of the administered dose was accounted for as excreted thiocyanate or intact glycoside. Following oral dosing, 89–97% of the administered dose was recovered.

A higher LD_{50} of 880 mg/kg body weight was determined for oral amygdalin in Wistar rats (Adewusi and Oke, 1985). However, when amygdalin was administered along with β-glucosidase a dose of 600 mg/kg body weight was fatal in all rats. Rats receiving 200, 400 or 600 mg/kg body weight of amygdalin (n = 2 per dose group), excreted 11.7%, 18.5% and 12.4% of the administered dose as intact glycoside in urine. Thiocyanate equivalent to 18.0, 11.2 and 7.8% of the administered dose was also excreted in urine in the 48 h following dosing. It should be noted that this study measured 'intact' amygdalin as enzymatically released benzaldehyde. It is likely that the excreted glycoside was prunasin, not amygdalin, as claimed by the authors. In contrast to other studies, administration of intraperitoneal amygdalin (300 mg/kg body weight) resulted in urinary excretion of intact glycoside (12.7–14.6% of administered dose) and thiocyanate (equivalent to 5.8–7.1% of the administered dose). The production of thiocyanate suggests that intracellular hydrolysis of amygdalin occurred to some extent.

Metabolism of amygdalin was studied in beagle dogs (n = 8) and Wistar rats (n = 8) following intravenous or oral administration (Rauws et al., 1982). This study was the first to use high-performance liquid chromatography (HPLC) for the analysis of intact cyanogenic glycosides and identified that the major glycoside excreted following oral administration was prunasin, not amygdalin. In beagles, 44–80% of the administered intravenous dose (500 mg) was recovered intact in urine during a 6-h period following administration. Using the ratio of amygdalin clearance to that of a reference compound (diatrizoate) and excluding data from a dog that managed to urinate outside the cage, the fraction of the administered dose excreted by glomerular filtration was estimated to be 97 ± 3%. Following oral administration (500 mg), only 0.6–1.2% of the dose was recovered from the urine as amygdalin, while 10–31% was recovered as intact prunasin. A similar pattern of excretion was seen in rats receiving 50 mg amygdalin. Following intravenous administration, 70 ± 4% of the dose was recovered in urine as intact amygdalin, while a further 6.6 ± 2.0% was recovered as prunasin. Following oral administration, 0.8 ± 0.5% of the dose was recovered in urine as intact amygdalin, while 39 ± 9% was recovered as prunasin.

The appearance of prunasin in rat (Sprague-Dawley) plasma following ingestion of amygdalin or peach seed decoction was studied (Chen et al., 2012). Amygdalin is the major cyanogenic glycoside in peach seed. Prunasin, but not amygdalin, was detected in rat plasma. The peak plasma concentration of prunasin (C_{max}) was greater (62.1 mg/L compared to 42.9 mg/L) and appeared earlier (45.3 min compared to 60.1 min) when rats received peach seed decoction, compared to amygdalin alone. It was suggested that this may be due to β-glucosidase in the peach seed decoction leading to earlier hydrolysis of amygdalin to prunasin.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to detect metabolites of amygdalin in rat urine following intraperitoneal administration (Ge et al., 2007). The parent compound and seven metabolites were detected and tentative structures assigned on the basis of molecular weight changes. No information is provided on the relative abundance of the different metabolites.

3.2.4. Prunasin

It should be noted that, while humans may be exposed to prunasin directly, exposure is more likely to be due to hydrolysis of amygdalin to prunasin in the gut.

Prunasin (100 mg) was given intravenously and orally to dogs (n = 2, approximately 10 kg body weight) (Rauws et al., 1982–83). The pharmacokinetics of prunasin were compared to amygdalin. Prunasin is extensively absorbed from the gastrointestinal tract (possibly even completely absorbed) and rapidly cleared by biotransformation in the liver. The absolute availability, calculated as the ratio of the plasma area under the curve (AUC) for oral compared to intravenous administration was about 50%, compared to 2% for amygdalin. No information was provided on cyanide formation from prunasin.

A mixture of prunasin and its diastereoisomer, sambunigrin, was produced for toxicity testing (Sakata et al., 1987). The LD_{50} was 560 mg/kg body weight in male Wistar rats. All animals died within 5 h. Rats in the second highest dose group (630 mg/kg) showed highest cyanide concentrations in the large intestine, followed by the small intestine. This was taken as confirmation that the hydrolysis of the cyanogenic glycosides occurs in the gastrointestinal tract. Approximately 30–45% of the administered dose was excreted intact in 24-h urine from surviving rats. The kinetics of the diastereoisomers appears to be similar.

3.2.5. Dhurrin

Female albino rats were fed sprouted sorghum containing 1.83 mg of bound cyanide (dhurrin) and 0.91 mg of free cyanide (Okoh et al., 1988). A proportion of the rats were killed after 5, 7 and 9 h and intestinal contents, intestinal wall materials, faeces, urine and plasma were analysed for dhurrin and free cyanide. Intact dhurrin was only detected in intestinal contents and urine, while free cyanide was

Table 5
Summary of human studies reviewed on the metabolism of cyanogenic glycosides.

Compound	Study type	Dose level (mg/kg body weight)	Reference
Food sources (apricot kernels, linseed, cassava, persipan paste)	Bioavailability as blood cyanide	1.7–27.2 mg cyanide equivalents	Abraham et al. (2016)
Linamarin (cassava)	Urinary excretion	1–2.5	Carlsson et al. (1999)
	Urinary excretion	NS	Hernández et al. (1995)
	Bioavailability as blood cyanide	NS	Oluwole et al. (2002)
Amygdalin	Blood and urinary amygdalin and thiocyanate following intravenous or oral administration	1.5 g/day	Ames et al. (1981); Moertel et al. (1981)
Linustatin/neolinustatin (linseed)	Plasma and urinary cyanide and thiocyanate	4.5 or 9 mg cyanide equivalents	Schulz et al. (1983)

NS: not stated.

detected in all materials tested. While the ratio of bound to free cyanide in the gastrointestinal tract suggests some hydrolysis of dhurrin occurred, intact dhurrin was present in urine. Unfortunately, the authors were unable to calculate the proportion of ingested dhurrin that was excreted unchanged.

3.3. Human studies

Table 5 summarises the human studies reviewed.

3.3.1. Comparative

A cross-over study design was used to assess the bioavailability of cyanide from consumption of apricot kernels, linseed, cassava or persipan paste (Abraham et al., 2016). Persipan paste is a confectionery preparation containing 47% bitter apricot kernels, 36% sugar and 17% water. Adult volunteers (n = 12) fasted overnight then consumed one of five food applications; amounts of apricot kernels, linseed, cassava or persipan paste to deliver a potential hydrogen cyanide dose of 6.8 mg or twice this amount of persipan paste. Blood cyanide was measured at 15-min intervals for up to 3 h, with the key measure being the maximum blood cyanide concentration, c_{max}. After a wash out period of at least two weeks, the process was repeated until all volunteers had received each of the five applications. Mean c_{max} values (μM) were 1.44 (persipan), 3.40 (double persipan), 6.40 (linseed), 15.5 (apricot kernels) and 17.0 (cassava).

This study differed from others, as the foods were consumed raw with foods thoroughly chewed before swallowing. Under these conditions, rapid hydrolysis by endogenous β-glucosidase appears to be the primary mode of cyanide production. The production of persipan paste involves heating to greater than 100 °C, likely to result in complete inactivation of the endogenous β-glucosidase. The much lower c_{max} for persipan paste compared to apricot kernels is likely due to relative slow microbial hydrolysis of amygdalin in the large intestine. The authors of this study suggested that the intermediate c_{max} values following consumption of ground linseed were due to a less effective β-glucosidase enzyme in this plant material. Hydrolysis of the cyanogenic glycosides present in linseed, linustatin and neolinustatin, is a two-step process, involving conversion of the gentiobiose sugar moiety to glucose, followed by hydrolysis of the glycoside. Although amygdalin, the primary cyanogenic glycoside present in apricot kernels also undergoes a two-step process, the structure of the intermediate cyanohydrin is quite different and is more favourable for cyanide formation.

A more diverse array of oral dosing scenarios was examined for a single volunteer, including five intake doses of linseed, three doses of persipan paste, two doses of potassium cyanide, two doses of amygdalin and two doses of linamarin. In addition, intake of persipan paste and amygdalin, delivering 6.8 mg of cyanide, was also carried out with co-administration of sweet almonds, as a dietary source of β-glucosidase.

For all applications, the c_{max} was dose-dependent, but not linear. Higher intake doses of potassium cyanide or cyanogenic glycosides produced proportionally greater increases in blood cyanide than lower doses. This finding is suggestive of a saturable detoxification mechanism for cyanide.

The c_{max} value from intake of potassium cyanide to deliver a potential dose of 6.8 mg of cyanide (20.1 μM) was virtually the same as for cassava (19.5 μM) and only slightly higher than for apricot kernels (15.4 μM). This suggests that the endogenous enzymes in these foods are able to release cyanide virtually quantitatively.

Co-administration of sweet almonds (10 g) with persipan paste or amygdalin resulted in approximately 2-fold and 3-fold increases in c_{max}, respectively. This suggests that the endogenous enzymes in sweet almonds are able to increase the cyanide generation from these sources, but not to the extent of the endogenous enzymes in cassava and apricot kernels.

3.3.2. Linamarin

Human volunteers (n = 15, 9 male, 6 female, 50–83 kg) consumed cassava flour mixed with water to form a porridge (Carlsson et al., 1999). Individuals consumed 275–650 g of porridge, representing 243–574 μmol of linamarin or 4–10 μmol/kg body weight. Intact linamarin was recovered in 24-h urine (mean = 21% of ingested dose, range 1–47%). In the second 24-h period following ingestion, much lower amounts of intact linamarin were recovered from urine (mean = 1%, range 0.5–4.0%).

A second group of human volunteers (n = 7, 6 male, 1 female, 55–96 kg) consumed cassava porridge (230–750 g), corresponding to 203–669 μmol of linamarin. In the first 48 h following ingestion, mean urinary excretion of intact linamarin was 28% (range 12–51%), with most excreted in the first 24 h (mean = 23%, range 9–39%). About half of total linamarin urinary excretion occurred in the first 8 h. Five-day urinary thiocyanate, from metabolism of generated cyanide equated to an average of 27% of ingested linamarin (range 1–46%). This left 3–68% of ingested linamarin unaccounted for.

A similar study was carried out in Cuba. Volunteers (n = 5, 2 male, 3 female, 31–42 years, 63–77 kg) consumed a serving of boiled cassava (mean = 480 g), with a mean cyanogenic glycoside content of 105 μmol of linamarin and 6 μmol of HCN (Hernández et al., 1995). Urinary excretion of linamarin peaked within the first 12 h and had fallen almost to baseline by 24 h. The mean total linamarin excreted was 28% of the ingested dose. Only very modest increases in urinary thiocyanate were observed, suggesting negligible exposure to cyanide liberated from linamarin.

Oluwole et al. measured blood and erythrocyte cyanide in 12 volunteers following consumption of a meal of *gari* (a cassava product) (Oluwole et al., 2002). No cyanide was detected in erythrocytes. Cyanide concentrations peaked in plasma by 9 h and had returned to baseline levels by 12 h. Total cyanide absorbed into the systemic circulation equated to 2–32% (mean 13%) of the ingested dose.

3.3.3. Amygdalin

Patients with advanced cancer (n = 6) received either intravenous or oral doses of amygdalin (Ames et al., 1981; Moertel et al., 1981).

Patients receiving intravenous amygdalin (4.5 g/m^2 per day for 21 days), had intact amygdalin detected in blood and in 24-h urine, accounting for 62–96% of the intravenous dose. Cyanide was essentially undetectable in whole blood, although a slight increase in plasma thiocyanate was observed in some patients. When patients received oral amygdalin (0.5 g three times daily), blood levels of intact amygdalin were much lower than following intravenous administration and only 8–32% of the ingested dose was recovered from 24-h urine. Peak blood cyanide levels were observed 1.5–2 h after dosing. There were also significant increases in blood thiocyanate, with peaks occurring approximately 48 h after dosing. It should be noted that the analytical method used to measure intact amygdalin measured benzaldehyde produced by hydrolysis, and would not have distinguished between amygdalin and prunasin.

3.3.4. Linustatin/neolinustatin

Volunteers ($n = 25$) received either single doses of 30 g of linseed or 15 g of linseed three times daily for 2 or 5 weeks (Schulz et al., 1983). One volunteer also received a single dose of bitter almonds (10 or 50 almonds) and potassium cyanide (3, 6 or 12 mg) on separate occasions. The linseed was claimed to contain 300 mg/kg HCN equivalents, while the bitter almonds contained 2500 mg/kg HCN equivalents.

Following ingestion of single doses of 30 g linseed (9 mg HCN equivalents), plasma HCN and thiocyanate were monitored for 180 min. A slight increase in plasma HCN was seen at 15 or 30 min, but concentrations generally returned to baseline by 1–2 h. No clear pattern in plasma thiocyanate concentrations was observed.

Volunteers receiving 15 g linseed (4.5 mg HCN equivalents) 3 times per day for 5 weeks were monitored weekly. No consistent pattern was seen in serum HCN concentrations, although mean thiocyanate concentrations increased throughout the trial.

In volunteers receiving 15 g linseed (4.5 mg HCN equivalents) 3 times per day for 2 weeks, plasma and urine thiocyanate concentrations were increased at the end of the trial compared to pre-trial levels.

Plasma cyanide increased to approximately 160 nmol/ml after 90 min in a volunteer consuming 50 bitter almonds (assuming almonds weigh approximately 1 g each, 50 almonds would represent a consumption dose of approximately 125 mg HCN equivalents) and returned to baseline after 7 h. When the same volunteer consumed 100 g of linseed (30 mg HCN equivalents) or 10 bitter almonds, plasma cyanide did not increase significantly above baseline. Ingestion of 3, 6 or 12 mg of potassium cyanide resulted in increases in plasma cyanide, peaking at approximately 20–30 min and retuning to baseline by 3 h.

While these studies do not inform the elucidation of the metabolism process for linseed cyanogenic glycosides, they do suggest a low availability of the cyanide in linseed.

4. Conclusions

Information on the metabolism of cyanogenic glycosides is fragmentary and no single source has examined the topic systematically. Information is available from *in vitro*, animal and human studies. Fortunately, information from these different sources contributes to a largely consistent picture of cyanogenic glycoside metabolism.

In the absence of endogenous β-glucosidase enzymes, two processes appear to contribute to the production of cyanide from cyanogenic glycosides; the proportion of the glycoside dose that reaches the large intestine, where most of the bacterial hydrolysis occurs, and the rate of hydrolysis of cyanogenic glycosides to cyanohydrin and cyanide.

It appears that some cyanogenic glycosides are actively absorbed in the jejunum by utilising the epithelial sodium-dependent monosaccharide transporter (SGLT1) (Wagner and Galey, 2003). A large proportion of ingested prunasin (30–100%) is absorbed intact, while almost no amygdalin is absorbed intact. Linamarin appears to be intermediate between the two, with human studies showing an average of approximately 20% of the ingested dose excreted intact in urine. It is unclear why prunasin is absorbed to a greater extent than linamarin, as both contain monosaccharide moieties, but it is possible that the other attached groups (phenyl and hydrogen for prunasin and methyl and methyl for linamarin) are able to influence the interaction of the glycoside with the SGLT1. On this basis it appears likely that dhurrin/taxiphyllin would be substantially absorbed in an intact form, as they also contain a monosaccharide group. Lotaustralin/linustatin/neolinustatin contain the disaccharide gentiobiose and are not likely to be absorbed to a great degree by this mechanism.

Two factors appear to influence the rate of cyanide production from cyanogenic glycosides due to bacterial β-glucosidase activity:

- The sugar moiety in the molecule. Cyanogenic glycosides with a gentiobiose sugar, amygdalin, linustatin, and neolinustatin, undergo a two stage hydrolysis, with gentiobiose initially being hydrolysed to glucose to form prunasin, linamarin and lotaustralin respectively.
- The stability of the intermediate cyanohydrin following hydrolysis by bacterial β-glucosidase. For example, hydrolysis of prunasin results in much more rapid cyanide production than hydrolysis of linamarin.

The cyanohydrin exists in equilibrium with the associated aldehyde or ketone and the literature suggests that the position of this equilibrium is largely driven by the stability of the ketone/aldehyde (Evans and Young, 1954; Lapworth and Manske, 1928, 1930). The stability of the aldehyde/ketone is predominantly due to the ability of substituent groups to stabilise the partial positive charge of the carbonyl carbon. Phenyl substituents will favour formation of the aldehyde/ketone and release of cyanide. Phenyl substituents with a hydroxyl group in the *para*-position will produce the most stable aldehydes/ketone (Lapworth and Manske, 1928).

On this basis, prunasin/sambunigrin and dhurrin/taxiphyllin that reaches the large intestine is likely to be rapidly converted to cyanide, due to these compounds containing a monosaccharide group and producing less stable cyanohydrins. The rate of conversion of amygdalin to cyanide will initially be slow, due to the need for the disaccharide to be hydrolysed to the corresponding monosaccharide, but will then be rapidly converted to cyanide due to the instability of the cyanohydrin formed.

Linustatin and neolinustatin, the glycosides found in linseed, will be the slowest cyanide producers due to the need for the gentiobiose moiety to be hydrolysed to glucose and the higher stability of the cyanohydrin formed (or the lower stability of the associated ketone). This is consistent with human studies that have shown a lack of increase in plasma cyanide following consumption of linseed (Schulz et al., 1983).

Animal studies, including a radiotracer study, found no intact cyanogenic glycoside in the faeces (Barrett et al., 1977; Carter et al., 1980; Jansz and Inoka Uluwaduge, 1997; Okafor and Maduagwu, 1999; Umoh et al., 1986). This suggests that the total ingested dose is absorbed, either intact or as HCN or as an unidentified metabolite. While similar faecal measurements were not made in human studies, there is evidence that up to 50% of the ingested cyanogenic glycoside may result in human cyanide exposure (Carlsson et al., 1999). However, it should be noted that cyanide exposure appears to vary markedly between individuals receiving similar doses of cyanogenic glycoside.

Approximately half the ingested dose of cyanogenic glycoside is unaccounted for in mass balance studies in animals and humans. Two possible mechanisms have been suggested for the fate of this cyanogenic material; hepatic metabolism of the intact glycoside to form an unknown metabolite that is subsequently catabolised, or conversion of hydrogen cyanide to β-cyanoalanine in a fast reaction, followed by conversion to asparagine.

While the overall impact of these metabolic factors is difficult to predict, several conclusions can be reached:

- In the absence of endogenous β-glucosidase enzymes, the toxicity of

cyanogenic glycosides will be less than the toxicity suggested by their theoretical hydrocyanic acid equivalents. This is due to intact glycoside being absorbed, stability of cyanohydrins and hepatic conversion of either intact glycoside, cyanohydrin or cyanide to stable, non-toxic compounds.

- The only studies that can substantially address issues of human metabolism of cyanogenic glycosides are human feeding trials. None of the trials conducted to date has succeeded in producing a full mass balance or accounted for the metabolic fate of the total ingested dose of cyanogenic glycoside. Such studies also show considerable person-to-person variability in metabolic indicators (excreted cyanogenic glycoside, blood cyanide) (Ames et al., 1981; Carlsson et al., 1999; Hernández et al., 1995; Moertel et al., 1981; Oluwole et al., 2002). This is likely to be due to factors such as the individual's gut microbial ecology and a better understanding of the impact of human gut ecology on cyanide production from cyanogenic glycosides is an important research question.

Most of the studies reviewed are now quite old and in many cases the analytical techniques used were non-specific. New studies, using chromatographic techniques to characterize and quantify metabolites of the cyanogenic glycoside would add to the picture suggested by the available evidence. Further studies employing radio-labelled analogues of the cyanogenic glycosides would also assist in identifying the full range of cyanogenic glycoside metabolites. The literature is also largely focused on amygdalin and linamarin and little information is available on cyanogenic glycosides such as dhurrin and taxiphyllin. In particular, dhurrin is present in an economically important crop (soy) and appears worthy of further study.

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