Please note that this opinion replaces the earlier version which contained an error in the 'conclusion and recommendation' section on page. 23.

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on

hydrocyanic acid in flavourings and other food ingredients with flavouring properties

Question number EFSA-Q-2003-145

Adopted on 7 October 2004

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SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food is asked to evaluate substances used as flavourings or present in flavourings or present in other food ingredients with flavouring properties. In particular, the Panel is asked to advise the Commission on the implications for human health of hydrocyanic acid (HCN) in the diet.

The Panel noted that cyanogenic glycosides present in plants, as sources of hydrocyanic acid, are relatively non-toxic until HCN is released. This can occur as a result of enzymatic hydrolysis by β-glucosidases following maceration of plant tissue, or by the gut microflora. Depending on the specific glycosides the hydrolysis products can be, besides sugar moieties and HCN, benzaldehyde (for amygdalin, prunasin, sambunigrin,), p-hydroxybenzaldehyde (for dhurrin) and acetone (for linamarin). The potential toxicity of a cyanogenic plant depends primarily on its capacity to produce HCN.

The Panel noted further that:

1. Following oral administration, HCN is readily absorbed and rapidly distributed in the body via the blood.

2. HCN absorbed from the gut is metabolically converted to the less toxic thiocyanate. Other detoxification pathways include combination with vitamin B_{12} or some sulphur-containing amino acids. Acute toxicity results when the rate of absorption of HCN is such that the metabolic detoxification capacity of the body is exceeded.

3. Cases of human intoxication and chronic neurological effects have occurred from the ingestion of processed plants. This is particularly apparent when processing practices change as a result of trading practices or uncertain food supply.

4. The cyanide ion inhibits enzymes associated with cellular oxidation and causes death through energy deprivation. The symptoms, which occur within a few minutes, may include constriction of the throat, nausea, vomiting, giddiness, headache, palpitations, hyperpnoea then dyspnoea, bradycardia, unconsciousness and violent convulsions, followed by death.

5. The occurrence of intoxication symptoms depends upon the rapidity of the increase in HCN concentration in the tissues. In the case of cyanogenic glycosides, the route of exposure, the nature of the cyanogenic compound, the dose, and the ability of the organism to detoxify cyanide determine the symptoms.

6. The available toxicity data show that cyanogenic glycosides from certain plants could produce acute toxic effects. Thus fatalities have occurred from e.g. consumption of stone fruit kernels.

7. The chronic uptake of HCN, in sub-acutely toxic doses, may be involved in the pathogenesis of certain conditions including disturbance of thyroid function and neuropathies. The thyrotoxic effects of cyanide depend on its conversion to the iodine antagonist, thiocyanate.

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

9. Several epidemiological studies in cassava-eating populations, which established an association between cyanide exposure and spastic paraparesis, amblyobia ataxia or tropical ataxia neuropathy (TAN) and possibly goitre have also been considered. However, the data are highly confounded by other nutritional and environmental factors. Adequate long-term toxicity studies in animals fed a diet containing HCN or cyanogenic glycosides are also lacking.

10. Limited data from the UK show that the average and high (97.5^{th} percentile) daily intake of HCN from its use in flavours or flavour ingredients were 46 and 214 μg/person, which correspond to approximately 0.8 and 3.6 μg/kg bw/day respectively.
Data from a Norwegian dietary survey show that the average and high (97.5^{th} percentile) daily intake of HCN among consumers amounts to respectively 95 and 372 μg/person or 1.4 and 5.4 μg/kg bw/day.

Cassava flour is used as a staple food mainly outside Europe; a consumption of 200 g/person would lead to an estimated intake level of 30 μg HCN/kg bw for a 60 kg adult. In accordance with the JECFA view such an intake would not be associated with acute toxicity. The highest level of HCN found in retail marzipan paste is 20 mg HCN/kg. Assuming on one sitting a person of 60 kg consumes 100 g marzipan containing such a level, that intake would be equivalent to 2 mg HCN or to 0.03 mg/kg bw.

The Panel concluded that the current exposure to cyanide from flavouring ingredients (97.5^{th} percentile) is unlikely to give rise to acute toxicity. For chronic exposure the overall data were not considered adequate to establish a numerical no-observed-adverse-effect level (NOAEL) or Tolerable Daily Intake (TDI) in humans. In view of the lack of adequate data on chronic toxicity, the Panel supports the continued application of limits for the presence of HCN in foods and beverages.

KEY WORDS
Hydrocyanic acid, Prussic acid, Cyanide, Amygdalin, Linamarin, Prunasin, Flavourings

BACKGROUND

In 1992, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated cyanogenic glycosides but could not estimate a safe level of intake for these compounds. However, JECFA concluded that a level of up to 10 mg HCN/kg food, as specified in the ‘Codex Standard for Edible Cassava Flour’, was not associated with acute toxicity (WHO, 1993).

In 2000 the Committee of Experts on Flavourings of the Council of Europe (CEFS) evaluated the safety of cyanogenic glycosides (CoE, 2000). Based on an overall toxicological assessment, but in particular based

on the data of studies on a Konzo-affected[1] population in the Democratic Republic of Congo, with a daily intake equivalent to 0.19-0.37 mg CN^-/kg bw/day, a TDI of 20µg CN^-/kg bw/day was set. CEFS proposed upper levels of CN^- of 0.5 mg/kg in food and 0.05 mg/kg in beverages with the exceptions of 0.5 mg/kg in stone fruit juices, 2 mg/kg in canned stone fruit and stone fruit preserves and purees, 50 mg/kg in marzipan and similar products, 10 mg/kg in almond and/or marzipan (or other similar products)-containing confectionery and baked goods, 40 mg/kg in "special" almond- and/or marzipan (or other similar products)- containing confectionery and baked goods e.g. "amaretti", "Dresdner Christstollen", "Schwarzbrötchen", chocolate enrobed marzipan, marzipan novelties and 0.5 mg/kg for every 1% alcohol by volume in alcoholic beverages. In plants hydrocyanic acid is present as cyanogenic glycosides. There are around 60 known cyanogenic glycosides found in over 2000 plant species.

In 1993 WHO derived a health-based guideline for (for both acute and long-term exposure) cyanide in drinking water of 0.07 mg/L (WHO, 2003).

Annex I B of EU Council Directive 98/83/EC on the quality of water intended for human consumption sets a maximum limit for cyanide of 0.05 mg/L (EC, 1998).

Current EU regulatory status

Annex II of Directive 88/388/EEC on flavourings sets the following maximum levels for hydrocyanic acid in foodstuffs and beverages to which flavourings or other food ingredients with flavouring properties have been added: 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 volume of alcohol in alcoholic beverages and 5 mg/kg in canned stone fruit. Hydrocyanic acid may not be added as such to foodstuffs (EEC, 1988).

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is asked to advise the Commission on substances used as flavourings or present in flavourings or present in other food ingredients with flavouring properties for which existing toxicological data indicate that restrictions of use or presence might be necessary to ensure safety for human health. In particular, the Committee is asked to advise the Commission on the implications for human health of hydrocyanic acid in the diet.

ASSESSMENT

Chemistry

Hydrocyanic acid (HCN), CAS-No: 74-90-8 (synonym, prussic acid); molecular mass 27.03, colourless gas or liquid with a characteristic odour.

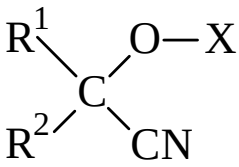
Cyanide (CN^-), CAS-No: 57-12-5 (synonym, isocyanide, cyanide anion); molecular mass 26.0.

The cyanogenic glycosides occurring in foods are amygdalin, dhurrin, linamarin, linustatin, lotaustralin, neolinustatin, prunasin, sambunigrin and taxiphyllin. From a flavouring point of view the major sources are those liberating benzaldehyde as a flavouring compound, namely amygdalin, sambunigrin and prunasin.

The general structure of the cyanogenic glycosides is schematised in Table 1.

[1] Konzo is a distinct form of a tropical myelopathy (spastic paraparesis). The disease has been attributed to dietary cyanide exposure from inadequately processed cassava.

Table 1. General structure of cyanogenic glycosides



Name	Formula Mol. mass CAS-number	R^1	R^2	X	Configuration	Occurrence*
Amygdalin	$C_{20}H_{27}NO_{11}$ 457.4334 29883-15-6	Phenyl	H	Gentiobiose	R	Almonds, Peach, Apricot, Prune, Cherry, Apple & Quince kernels
Linamarin	$C_{10}H_{17}NO_6$ 247.2474 554-35-8	Methyl	Methyl	Glucose	-	Cassava, Lima bean, (Flax seed)
Prunasin	$C_{14}H_{17}NO_6$ 295.29 99-18-3	Phenyl	H	Glucose	R	Ferns, e.g. Bracken fern, Rowanberries
Linustatin	$C_{16}H_{27}NO_{11}$ 409.39 72229-40-4	Methyl	Methyl	Gentiobiose	-	Flax seed, Cassava
Lotaustralin	$C_{11}H_{19}NO_6$ 261.272 534-67-8	Methyl	Ethyl	Glucose	R	Lima bean, (Cassava), (Flax seed)
Neolinustatin	$C_{17}H_{29}NO_{11}$ 423.42 7229-42-6	Methyl	Ethyl	Gentiobiose	R	Flax seed
Sambunigrin	$C_{14}H_{17}NO_6$ 295.29 138-53-4	Phenyl	H	Glucose	S	Elderberries
Taxiphyllin	$C_{14}H_{17}NO_7$ 311.29 21401-21-8	p-Hydroxy-phenyl	H	Glucose	R	Bamboo shoot
Dhurrin	$C_{14}H_{17}NO_7$ 311.29 499-20-7	p-Hydroxy-phenyl	H	Glucose	S	Durra, (Sorghum)

* minor sources are indicated between parenthesis

On hydrolysis, one gram of the respective cyanogenic glycosides can liberate the following quantities of HCN: amygdalin, 59.1 mg HCN (equivalent to 56.9 mg CN^-), linamarin 109.3 mg HCN (equivalent to 105.2 mg CN^-) and prunasin 91.5 mg HCN (equivalent to 88.1 mg CN^-).

The release of HCN from cyanogenic glycosides can occur as a result of enzymatic hydrolysis by β-3-glucosidases following maceration of plant tissue, or by the gut microflora. Depending on the specific glycosides the hydrolysis products can be, besides glycosidic portions and HCN, benzaldehyde (for amygdalin, prunasin, sambunigrin,), p-hydroxybenzaldehyde (for dhurrin) and acetone (for linamarin). The potential toxicity of a cyanogenic plant depends primarily on its capacity to produce HCN.

The cyanogenic glycosides differ considerably in bioavailability, but those absorbed intact from the gut are not biotransformed to HCN by mammalian enzymes. As a consequence cyanogenic glycosides are relatively non-toxic in germ free animals.

Exposure

The sources and potential levels of HCN liberated from cyanogenic glycosides in edible plants are given in Table 2.

Table 2. Sources and levels of HCN in edible plants

Plant	Parts with HCN	Level of HCN (mg/kg) (free and bound)	Type of glycoside	Reference
Cassava	Root (sweet variety)	10-20	Linamarin	Ogunsua, 1989
	Root (bitter variety)	60-200	Linamarin	Ogunsua, 1989
		55		Lindner, 1974
		15-1120		Rosling, 1987
Lima bean	Seed[1]	200	Linamarin	Gypta, 1987; Holzbecher, 1984
White American variety		100		Lindner, 1974; Conn, 1973
Burma variety		2000		Lindner, 1974; Conn, 1973
Black Puerto Rican variety		3000		Lindner, 1974; Conn, 1973
Garden bean	Seed	20	Linamarin	Lindner, 1974
Bitter almond	Seed, kernel	2900-3100	Amygdalin	Sturm and Hansen,1967
		300-3400		Lindner, 1974; WHO, 1993
	In almond oil	800-4000		Rosling, 1987; Gypta, 1987
Apricot	Seed, kernel	120-4000	Prunasin	Gypta, 1987; Holzbecher, 1984
Peach	Seed, kernel	470	Prunasin	Lindner, 1974
Pea	Seed	20		Lindner, 1974
Soya bean	Protein	70-30 µg/kg		Honig, D. *et al*., 1983

Plant	Parts with HCN	Level of HCN (mg/kg) (free and bound)	Type of glycoside	Reference
	Shell	1240 µg/kg		
Linen flax	Seed	>500	Linamarin	Honig, D. *et al*., 1983

1 According to Gypta (1987) and Holzbecher (1984), the normal level in Lima bean seeds is 144-167 mg HCN/kg; The level associated with fatal human poisoning amounts to 2100-3120 mg HCN/kg.

A summary of the major sources of the intake of HCN from flavourings and food ingredients with flavouring properties is given in Table 3.

The overall intakes of HCN by the average and high (97.5th percentile) consumer have been calculated based on data from the 'Dietary and Nutritional Survey of British Adults' (Gregory *et al*., 1990) and maximum levels in foods according to the proposed Council of Europe limits. The mean and high daily intakes reported (CoE, 2000) were 46 and 214 µg/person, which correspond to 0.7 and 3.3 µg/kg body weight/day.

A similar calculation on the intake of HCN as the British one was done based on data from the Norwegian dietary survey NORKOST 1997 (Johansson and Solvoll, 1999) and the maximum levels in foods according to the proposed Council of Europe limits. The average and high (97.5th percentile) daily intakes among consumers were 95 and 372 µg/person, which correspond to 1.4 and 5.4 µg/kg body weight/person.

Table 3. Compilation of the major sources of HCN in food ingredients with flavouring properties*

Type of product	Level of HCN in food (free and bound)	Reference
Ground almonds (powder)	1.4 mg/kg	Anon., 1975
Marzipan and other similar products made from apricot kernels (in the UK different grades of marzipan contain different concentrations of HCN)	15-20 mg/kg retail; 30-35 mg/kg (higher grade, manufacturing); ~ 50 mg/kg (baker's raw paste)	Schmidt, 1977
Marzipan novelties Almond paste	<0.8 mg/kg 3.0 mg/kg	Schmidt, 1977

Type of product	Level of HCN in food (free and bound)	Reference
Cherry juice	0.9 – 12 mg/L 0.6 – 1.3 mg/L 0.5 – 1.0 mg/L	Stadelmann, 1976 Schmidt, 1977 Eid & Schmidt, 1978
Plum juice	0.33 – 1 mg/L	Stadelmann, 1976, Schmidt, 1977
Apricot juice	0.3 – 7.8 >0.1 mg/L	Stadelmann, 1976 Schmidt, 1977
Peach juice	2.3 – 5.9 mg/L	Stadelmann, 1976
Stone fruit preserves	typically 0.18 mg/kg	Karkocha *et al*, 1992
Canned stone fruit	up to 4 mg/kg <0.01-0.02 mg/kg	Voldrich & Kyzlink, 1992 Misselhorn, 1976
Kirsch (distilled from cherries with up to 61% alcohol)	<10 mg/L	not stated
Calvados, Poire Williams (distilled from pears, with up to 40% alcohol)	<0.5 mg/L)	not stated
Stone fruit brandies	<3 mg/L (80% of the samples had undetectable levels of HCN)	Schmidt, 1977, 1977
Almonds and/or marzipan (or other similar products)–containing confectionery and baked goods	up to 40 mg/kg; median 44 mg/kg in amaretti	Corradi & Micheli, 1982
Chocolate enrobed marzipan	1.3 mg/kg	Schmidt, 1977

* Data from CoE (2002)

Acute exposure to HCN

The highest level of HCN found in retail marzipan paste is 20 mg HCN/kg. Assuming on one sitting a person of 60kg consumes 100 g marzipan containing such a level, that intake would be equivalent to 2 mg HCN or to 0.03 mg/kg bw.

Absorption, distribution, metabolism and excretion of cyanide and cyanogenic glycosides

Absorption, distribution and excretion of cyanides

Following oral administration, cyanides, at the physiological pH of the stomach, form predominantly HCN, which, can rapidly penetrate mucous, and cell membranes. Only 1.6% of HCN is dissociated at pH 7.4 and therefore the absorbed cyanides (HCN) readily diffuse through cell membranes. About 99% of the absorbed

HCN binds to methaemoglobin in erythrocytes; it is later converted in the liver to less toxic metabolites such as thiocyanate (SCN^-) (Askar, 1983, Frankenberg *et al*., 1975). The concentration of cyanide is therefore higher in erythrocytes than in plasma. At normal physiological levels the total body methaemoglobin of an adult human can bind approximately 10 mg of HCN. The rate of spontaneous detoxification of HCN by rhodanese (thiosulphate-cyanide-sulphurtransferase) in humans is about 1µg HCN/kg/min (Schultz, 1984).

The pharmacokinetics of $^{14}CN^-$ and $S^{14}CN^-$ were investigated in rats that had been exposed to these agents in the diet, for 3 weeks. All tissues contained radioactivity 9 h after intraperitoneal injection of $^{14}CN^-$; the highest level of radioactivity was found in the stomach (18%). Eighty per cent of this activity was in the form of thiocyanate. At this point 25% of the dose had already been eliminated in the urine and 4% in the expired air. When $S^{14}CN^-$ was given *per os* to rats with elevated plasma thiocyanate levels due to prior oral exposure to cyanide, most of the activity was eliminated in the urine and only small amounts were found in the faeces. These two studies indicated the existence of a gastrointestinal circulation of thiocyanate (Okoh & Pitt, 1981).

The excretion of a single oral dose of ^{14}C-labelled cyanide in urine, faeces and expired air was studied in rats (12 animals/group) pre-treated orally for 6 weeks with either unlabelled KCN (5 mg KCN/rat/day equivalent to 2.01 mg CN^-/rat/day*)* or with a control diet. Urinary excretion was the main route of elimination of ^{14}C-labelled cyanide in these rats, accounting for 83% of the total excreted radioactivity at 12 h and 89 % of the total excreted radioactivity at 24 h. The major metabolite of cyanide excreted in urine was thiocyanate, and this metabolite accounted for 71% and 79% of the total urinary activity after 12 h and 24 h, respectively. Only 4% of the mean total activity excreted was found in expired air after 12 h, and this value did not change after 24 h. Of the total activity in expired air in 24 h, 90% was present as carbon dioxide and 9% as cyanide. When these results were compared with those observed for fed the control diet, it was clear that the mode of elimination of cyanide carbon was altered in neither urine nor breath by chronic intake of cyanide (Okoh, 1983).

Biotransformation of cyanide

In humans, HCN is detoxified in three ways: (i) by conversion, in the liver, of cyanide to thiocyanate by rhodanese [thiosulphate-cyanide sulphur transferase, EC. 1.8.1.1], (ii) by direct chemical combination of cyanide with sulphur in the form of an amino acid (di-cysteine) with the formation of 2-aminothiazoline-4-carboxylic acid and cysteine; (iii) by combination of cyanide with hydroxycobalamin (*in vivo* or as a therapeutic expedient) to form cyanocobalamin (vitamin B12) (Askar, 1983; Ludwig *et al*., 1975; Freeman, 1988; US-DHHS, 1997). Thiosulphate and 3-mercaptopyruvate can act as sulphur donors, but free cystine or cysteine cannot. The enzyme (rhodanese) contains an active disulfide group that reacts with the thiosulphate and cyanide. Detoxification is therefore affected by the presence of nutritional factors, such as sulphur-containing amino acids and vitamin B12.

Since the enzyme rhodanese, which is usually localised in the mitochondria in different tissues, is relatively abundant, but in sites that are not readily accessible to thiosulphate, the limiting factor for the conversion of cyanide is thus thiosulphate (EPA, 1990). This provides an explanation for the action of exogenous thiosulphate in the treatment of cyanide poisoning.

The overall rate of *in vivo* detoxification of cyanide may be influenced by several minor reactions. Cystine may directly react with cyanide to form 2-imino-thiazolidine-4-carboxylic acid, which is excreted in saliva and urine. Traces of hydrogen cyanide may be found in expired air, saliva, sweat and urine. A minor amount may be converted into formic acid, which may be excreted in urine or participate in the metabolism of one carbon compounds.

One other detoxification route is the combination of cyanide with hydroxy-cobalamin (vitamin B_{12a}) to form cyanocobalamin (vitamin B_{12}) that is excreted in urine and bile. It may be reabsorbed by the intrinsic factor

mechanism at the level of the ileum allowing effective re-circulation of vitamin B_{12}. Methaemoglobin effectively competes with cytochrome oxidase (see below) for cyanide and the formation of methaemoglobin from haemoglobin, affected by sodium nitrite or amylnitrite, is exploited in the treatment of cyanide poisoning (EPA, 1990).

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

Effect of cyanide on enzymes and other biochemical parameters

Cyanide causes a decrease of the utilisation of the oxygen in the tissues, producing a state of histotoxic anoxia. This is achieved through the inactivation of tissue cytochrome oxidase by cyanide, which combines with Fe^{3+}/Fe^{2+} contained in the enzyme. The enzyme-cyanide complex dissociation constant has been found to be $1x10^{6}$ and $1x10^{4}$ (moles/L) for the oxidised and reduced form of the enzyme, respectively.

It has been pointed out that cyanide can inhibit several other metallo-enzymes containing for the most part iron, copper or molybdenum (e.g., alkaline phosphatase, carbonic anhydrase) as well as enzymes containing Schiff's base intermediates (e.g. 2-keto-4-hydroxyglutarate aldolase). The effect of sublethal doses of cyanide on the metabolism of glucose in mice has been studied using radio-respirometric techniques (Solomonson, 1981). 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 apparently activates glycogenolysis and shunts glucose to the pentose phosphate pathway decreasing the rate of glycolysis and inhibiting the tricarboxylic acid cycle (EPA, 1990).

Absorption, distribution and excretion of cyanogenic glycosides

In rat

Wistar rats (4 animals/sex/group) were given either single oral or intravenous doses of 50 mg amygdalin/rat after an overnight fast. After intravenous administration 70% and after oral administration 0.8% of the dose was excreted in the urine as unchanged amygdalin. The fraction excreted as prunasin after intravenous administration was 6.6% whereas it was 39% of the total dose after oral administration. This suggests that amygdalin is biotransformed at the site of absorption. There was no sign of toxicity (Rauws *et al*. 1983).

In a toxicokinetic study, 10 male rats (100-120 g) were given 50 mg linamarin in 0.5 ml of water by stomach tube. Seven rats died within 4 h. In a second trial 6 male rats were given 30 mg linamarin. Following dosing, urine and faeces were collected after 24, 48 and 72 h and heparinised blood samples were taken from the optic vein or the lateral tail vein. Blood samples were taken after 30, 40, 60, 80 and 100 min. and at 2, 4, 8, 24 and 48h. No intact linamarin was detected in faeces or blood of the rats dosed 30 mg (300mg/kg bw.). In the faeces of the three surviving rats dosed 50 mg (500 mg/kg bw.) also no linamarin was detected. Linamarin was excreted in the urine at a level of 5.65 mg (cumulated after 72 h.) along with 0.823 mg of thiocyanate. These findings indicate that a considerable proportion of the linamarin was absorbed intact and was partially hydrolysed. It could not be determined however, whether breakdown occurred in the intestinal tract or within the body of the rat (e.g. in the liver). Possibly both alternatives may have occurred. (Barrett *et al.*, 1977).

In a study with isolated perfused rat liver, Strugala and Elbers (1984) demonstrated that cyanoglycosides require gut microbial flora for their metabolism and that they are not metabolised by mammalian cells.

Linamarin, at a dose level of 300 mg/kg bw, was administered in the feed to a group of Wistar rats (60 rats, 10 rats/group) maintained on vitamin B_2-deficient, -sufficient and -excess diets for 5 weeks and to another group of kwashiorkor rats. Free and total cyanide, intact linamarin and thiocyanate levels were estimated in urine and faeces obtained at 0, 24, 48 and 72 h periods and in blood samples obtained 72 h after the compound had been administered. No cyanide or intact linamarin could be detected in the faeces samples. Rats on vitamin B_2-sufficient and B_2-excess diets excreted higher levels of total and free cyanide in the urine than the vitamin B_2-deficient group.

Most of the linamarin was degraded after 24 h. The rate of breakdown of the glycoside within the first 24 h was slowest for zero and half normal vitamin B2 status rats as evidenced by appearance of the glycoside in large quantities in the urine. The kwashiorkor rats, on the other hand excreted less thiocyanate than the controls. In addition, their control group excreted most of the thiocyanate in the first 24h, whilst the kwashiorkor rats excreted most of the thiocyanate in the first 48 h. Dietary protein deficiency prolongs the time of metabolism and hence increases the toxicity of cyanogenic glycosides in the body (Umoh *et al*, 1986).

In dog

Beagle dogs (4 animals/sex/group) were administered 500 mg amygdalin in 10 ml solution either intravenously or orally after an overnight fast. After intravenous administration the major part (71%) of the dose was recovered in the urine after 6 h. The fraction of the dose excreted by glomerular filtration was calculated using the ratio of diatrizoate (which was administered simultaneously) clearance to amygdalin clearance, showing that 97 % of the amount of amygdalin to be expected was recovered from the urine. The result of the experiments after intravenous administration were analysed assuming a two-compartment model. The distribution half-life time $T_{1/2}$ α was 0.10 h. and the elimination half-life time $T_{1/2\beta}$, 0.57 h. No prunasin was detected in urine (detection limit (= 0.2 % of dose). After oral administration of amygdalin a very low maximal plasma level was found after approximately 0.75 h. Only 2.3% of the amygdalin was available systemically (absolute bioavailability). Prunasin was found in the plasma and urine. In the urine collected during 6 h after amygdalin administration only about 1% of the dose was recovered unchanged whereas 21 % of the dose was identified as prunasin (Rauws *et al*, 1982).

Prunasin was administered intravenously and orally in 100 mg doses to female dogs (2/group; 10 kg) after an overnight fast. The results of the experiment after intravenous administration were analysed assuming a two-compartment model and compared to those previously obtained with amygdalin. The distribution half-life time $T_{1/2\alpha}$ was 0.08 h and elimination half-life time $T_{1/2\beta}$ was approximately 0.64 h. Prunasin was absorbed to a large extent after oral administration. The absolute bioavailability after oral administration was 50 % of the dose administered. The volume of distribution (0.34 L/kg) and the clearance (0.55 L/kg.h) were larger than those of amygdalin (respectively 0.19 L/kg and 0.39 L/kg.h). The oral bioavailability of prunasin is considerably greater than that of amygdalin, which was hardly (2.3 %) absorbed unchanged (Rauws *et al*, 1983).

In humans

In humans, studies have shown that amygdalin is broken down to HCN, benzaldehyde and glucose by enzymes found in gut bacteria, but not intracellularly. Animal and human tissues contain no significant concentrations of β-glucosidase, the only known activating enzyme of hydrolysis of cyanogenic glycosides *in vivo*. As a result, metabolic breakdown of the compound does not occur (Dorr and Paxinos, 1978).

The cyanide yielding capacity of insufficiently processed cassava is probably due to linamarin, or an intermediate breakdown product, from which cyanide may be released in the gut by action of microbial enzymes. Significant amounts of linamarin are observed in the urine after consumption of insufficiently processed cassava as well as after consumption of other plants containing linamarin. These results indicate that linamarin, if not metabolised in the gut, will be absorbed and excreted in the urine without causing exposure to HCN. About 80 % of ingested cyanide will be turned into thiocyanate and is excreted in the

urine after a short period (Rosling, 1987).

Toxicity studies

Acute toxicity of cyanides

Lethal oral doses for HCN and cyanide have been reported in the literature (Table 4).

Table 4. Acute toxicity studies with cyanide (Conn, 1979a)

Species	Route of intake	Acute toxicity		References
		LD_{50} (mg/kg bw)	LD_{50} (mg CN^-/kg bw)	
HCN				
Mouse	i.v.	0.99	0.95	EPA, 1990
Rat	i.v.	0.81	0.78	EPA, 1990
Guinea pig	i.v.	1.43	1.38	EPA, 1990
Rabbit	i.v.	0.66	0.64	EPA, 1990
Cat	i.v.	0.81	0.78	EPA, 1990
Dog	i.v.	1.34	1.29	EPA, 1990
Monkey	i.v.	1.30	1.25	EPA, 1990
KCN				
Mouse	s.c.	6.0	2.41	WHO, 1965
	i.v.	2.5	1.00	WHO, 1965
Rat	oral	10-15	4.02-6.03	WHO, 1965
	i.v.	2.5	1.00	WHO, 1965
Dog	oral	5.3	2.13	WHO, 1965

Hydrocyanic acid and cyanides combine in the tissues with cytochrome oxidases, the enzymes associated with cellular oxidation. They thereby render oxygen unavailable to the tissues and cause death through anoxia. The organ the most sensitive to cyanide toxicity is the brain and death is believed to result from central nervous system depression by inhibition of cytochrome oxidase in the brain (NTP, 1993). HCN does not combine easily with haemoglobin, but does combine readily with methaemoglobin to form cyanmethaemoglobin.

The symptoms that may occur are: constriction of the throat, nausea, vomiting, giddiness, headache, palpitations, hyperpnoea and dyspnoea, bradycardia, unconsciousness and violent convulsions followed by death. Similar effects occur more slowly following exposure to cyanide salts (Reynolds *et al*., 1989; Sax, 1984).

Acute toxicity of cyanogenic glycosides

A dose of 25 mg linamarin (250 mg/kg bw) fed to rats (100-120g bw) caused clinical signs of toxicity, including apnoea, ataxia and paraparesis. These symptoms were very marked in the absence of methionine supplementation; fifty percent of the rats died within 4 h. In the presence of adequate methionine supplementation, 10 % of the rats died and about 40 % showed no signs of toxicity. The activity of Na^+K^+ dependent ATPase was reduced in much the same way as it was by the glycoside, digitalis (reviewed by Oke, 1980).

In a toxicokinetic study 7 out of 10 rats (100g 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 (90g bw) produced signs of cyanide intoxication in a large percentage. Within 1h after dosing the following signs appeared: dyspnoea, hyperpnoea, ataxia, tremors and hypothermia. Two animals dosed with 140 mg/kg bw and one animal dosed with 120 mg/kg bw died within 2 hours of dosing. The signs of poisoning were greatly reduced or gone within 3 hours after treatment in the surviving animals. No relationship between length of intoxication and dose was observed (Frakes *et al*, 1985).

The LD_{50} for linamarin in rat (sex not indicated) was found to be 450 mg/kg bw via oral administration whereas it was 20,000 mg/kg bw, parenterally (Oke, 1979). Solomonson (1981) reported a parenteral LD_{50} for amygdalin in mouse (sex not indicated) of 0.1mM kg/bw. Hamsters have been reported to be more susceptible than rats for the acute toxic effects (Willhite, 1982).

Short-term toxicity with cyanides

In rat

In a 13-week toxicity study (Leuschner *et al*., 1989b), male Sprague-Dawley rats (30 rats/group) were administered KCN in the drinking water. The dose levels were 40, 80 and 160/140 mg KCN/kg bw/day. Three control groups were used, one given normal drinking water *ad libitum*, a "paired drinking" group (parallel to the high dose level KCN) and a group receiving drinking water with 10 % ethyl alcohol. In addition one group received drinking water with KCN (80 mg/kg bw/day) and 10 % alcohol. Behaviour, external appearance, body weight, food consumption (daily) and drinking water consumption (twice weekly) were recorded frequently. Extensive haematological, clinical chemical (in serum) and urine analyses were carried out in 5 animals per group in week 6 and week 13. Autopsy and macroscopy were performed after 13 weeks (20 animals/group) and 11 organs were weighed. Histopathological examination was performed in brain, kidneys, heart, liver and testes of these animals. In addition, thyroids of the control, the "pair drinking" control and the high dose group (160 mg/kg bw for 11 weeks, 140 mg/kg bw from week 12 because of observed reduced body weight gain, reduced water consumption and mortality) were examined.

There was a clear indication that reduced food consumption and body weight in the KCN groups were caused by a decrease in water consumption due to a decreased palatibility. Urinalyses revealed a dose related higher level of protein for the animals receiving KCN. Several changes in absolute organ weights were seen in the 160/140 mg KCN/kg bw group. Relative weights of organs were very slightly increased in the 40 mg, slightly increased in the 80 mg and clearly increased in the 160/140 mg KCN/kg bw groups. The thymus weight was, however, reduced in the high dose group. Histopathological examination revealed no indication of damage to the brain, heart, liver, testes, thyroids or kidneys due to treatment with KCN (Leuschner *et al*., 1989b).

In a 13-week study conducted by the National Toxicology Program (NTP, 1993), 10 animals (F344/N rats/sex/group) received 0, 3, 10, 30, 100 or 300 mg/L NaCN/day in drinking water. The achieved doses were 0, 0.3, 0.9, 2.7, 8.5 and 23.6 mg NaCN/kg bw/day in the males and 0, 0.3, 1.0, 3.2, 9.2 and 23.5 mg NaCN/kg bw/day in the females. The final mean body weights and body weight gains of males in the 10 and 30 mg/kg groups were slightly less than those of the controls. There were no apparent differences between the final mean body weights and body weight gains of exposed and control females. No clinical signs attributable to NaCN exposure were observed. In the 100 and 300 mg/L dose groups of both males and females, water consumption was reduced by more than 10% compared to that of the control groups. Changes in haematology were minor and sporadic and were not considered to be clinically significant. Decreases in urine volume and increases in urine specific gravity occurred in supplemental rats in the 300

mg/kg group at all time points and in the 100 mg/kg group on day 8. These changes were consistent with the observed decreases in water consumption and with subsequent decreases in urine output, suggesting a palatability problem.

Increases in urinary thiocyanate occurred in treated rats at all but the 3 and 10 mg/L exposure levels on days 22 and 88 and all but the 3 mg/L exposure level on day 43. Changes in urinary pH, sorbitol dehydrogenase, and N-acetyl-β-D-glucosaminidase were minor and not exposure related; these changes were not considered to be clinically significant. There were no treatment-related gross or histopathological lesions in rats of either sex. Cyanide treatment caused a slight reduction in the cauda epididymal weight of treated male rats. The number of sperm heads per testis was reduced in the 300 mg/L group compared to the controls and sperm motility was marginally reduced in treated animals. The authors suggest that sub-chronic exposure to low dose of cyanide may produce mild, but potentially biologically significant, adverse effects on the male reproductive tract (NTP, 1993).

In mouse
In a similar 13 weeks study as that with rats (NTP, 1993), NaCN was administered in the drinking water, at the same concentration as above, to 10 animals (B6C3F1 mice/sex group) in the drinking water. The achieved doses were 0, 0.5, 1.8, 5.1, 16.2 and 45.9 mg NaCN/kg bw/day in males and 0, 0.6, 2.1, 6.2, 19.1 and 54.3 mg NaCN/kg bw/day in females. The final mean body weights of males and females in the 3 mg/kg groups and males in the 30 mg/kg group were slightly greater than those of the controls, the final mean body weight of females exposed to 300 mg/kg was reduced compared to the control. No treatment-related clinical signs were noted. Water consumption by males and females in the 100 and 300 mg/kg groups was lower than that of the controls. Differences in absolute and relative organ weights of male and female mice were sporadic and not considered related to cyanide toxicity. A few changes in haematology or clinical chemistry were observed; these were minimal and were not considered to be biologically significant. There were no treatment-related gross or histopathological lesions in mice of either sex. Cyanide treatment caused a slight reduction in the cauda epididymal weight of mice in the 300 mg/kg group compared to the controls and sperm motility was marginally reduced in the treated animals (NTP, 1993).

Short-term toxicity with cyanogenic glycosides

In rat
Albino female rats (10 animals/group) were fed *ad libitum,* during 14 weeks, five diets; respectively, a normal laboratory diet (control), a 50 % gari diet (Nigerian preparation of cassava), a raw cassava diet, a diet containing 5 g KCN/100g and a diet containing 10 g KCN/l00g. The 50 % gari diet caused no significant biochemical and haematological changes in the female rats, whereas for both the raw cassava diet and the KCN diets a decrease in haemoglobin, PCV, total serum protein concentration and T_4 concentration was observed. In both the 50 % gari diet group and to a greater extent in the other treatment groups the serum thiocyanate levels were increased. Except for the 50 % gari group, the other treated groups showed, instead of a gain, a loss of body weight (Olusi *et al*., 1979).

In guinea- pig
In a 24-day toxicity experiment, guinea-pigs (Duncan-Hartley, weight 200-250 g, 8 animals/group) were divided in three groups: a control group receiving daily a 10% sucrose solution, two test groups dosed daily with respectively 10 mg laetrile (amygdalin) or 8 mg KCN/kg bw/day dissolved in 10% sucrose solution with, and without ascorbic acid (100 mg).
In a further 24-day experiment, three groups of animals (8 animals/group) were dosed orally respectively with 10% sucrose solution (control), ascorbic acid (300 mg), ascorbic acid (300 mg) plus cysteine (10 mg). After 24 hours an oral dose of KCN (8 mg/kg bw) following an overnight fast was administered. The authors estimated the lethal dose of KCN for this strain of animals to be 10 mg/kg while 8 mg/kg was non-lethal.

Daily oral administration of laetrile (10 mg) without or with ascorbic acid had no significant effect on the body weight and liver weight. However, treatment with laetrile alone for 4, 16 and 24 days respectively, resulted in a significant increase in urinary levels of thiocyanate. The increase was less in animals treated with vitamin C.
In guinea pigs treated with 8 mg KCN/kg bw toxic effects were seen as evidenced by slight tremors in 3 of the 8 animals, which recovered within 5 minutes. All animals in the KCN group, which were supplemented with ascorbate, showed severe tremors, motor ataxia, bizarre neuromuscular manifestations and rhythmic head movements. The toxicity of KCN increased with the increase in vitamin C, whereas urinary excretion of thiocyanate decreased.

The author concluded that in guinea pigs, conjoint use of laetrile and ascorbic acid (in large doses) decreases the detoxification of cyanide derived from laetrile through diminishing the availability of cysteine, but not impairing hepatic rhodanese activity, which is involved in the detoxification of cyanide to thiocyanate. These results agree with the symptoms of a sublethal dose of KCN toxicity manifested by the animals. The studies, therefore, indicate that individuals taking large doses of ascorbic acid concurrently with laetrile may be subject to self-poisoning (Basu, 1983).

In chicken
In two feeding experiments (63 or 56 days), one-day-old broiler chickens (male and female mixed) were fed a diet containing 0, 10, 20 and 30 % cassava, respectively. The animals were studied for haematological and histopathological effects. The cassava diet studied in the first experiment consisted of a high-cyanide-containing cassava root meal (CRM) supplying 300 mg of total cyanide/kg, most of it in the form of cyanogenic glycosides. The cassava diet in the 2nd experiment also contained cassava foliage meal (CFM) supplying 156 mg total cyanide/kg. In the 1st experiment 26 chickens per group were used and in the 2nd experiment 160 chickens for the cassava groups and 80 for the control group were used. No changes in the haematological parameters due to cassava were seen.

Addition of up to 30 % CRM did not adversely affect broiler survival, performance or feed efficiency, but the inclusion of CFM to the experimental diets increased mortality, decreased weight gain and decreased feed efficiency. In both experiments, increased quantities of dietary cassava cyanate were associated with increased ($P<0.05$) blood serum thiocyanate concentrations. Histopathological examination of thyroid, liver and kidney revealed no appreciable alterations due to the cassava feeding, however there was no conclusive evidence of cyanide or thiocyanate effects on thyroid activity. Aflatoxin contamination appeared to have contributed to the high mortality rate associated with CFM diets. The results showed that broiler chickens were tolerant to relatively high levels of dietary cyanogenic glycosides (Gomez *et al*, 1988).

In dog
Groups of six dogs were fed 100g/kg bw/day of a rice-based control diet, a cassava (gari) diet designed to release 10.8 mg HCN/kg feed or a rice-based diet supplemented with NaCN, at a level sufficiently high to provide 10.8 mg HCN/kg feed. Thus, the animals in the rice+NaCN and gari groups received a cyanide dose of 1.08 mg/kg bw/day. The exposure period lasted for 14 weeks.

The gari and rice+cyanide diets caused an increase in plasma thiocyanate and urinary thiocyanate. Both treated diets caused an increase in urinary protein excretion compared to the controls but this was much higher in the cassava treated group. The cassava diet caused generalised congestion and haemorrhage, periportal vacuolation of the liver, swelling vacuolation and rupture of the epithelial cells of the proximal convoluted tubules of the kidney, myocardial degeneration and adrenal gland degeneration. In the testes abnormal germ cells in the seminiferous tubules were occasionally seen as well as seminiferous tubules,

which were denuded of germ cells down to the basement membrane but with shreds of Sertoli cells being apparent. Spermatogenesis, however, appeared to be normal since the percentage of round tubules in stage 8 of the spermatogenic cycle was not significantly different from that of the control dogs.

The rice+cyanide caused nephrosis and a significantly reduced relative frequency of testicular tubules in stage 8 of the spermatogenic cycle ($P<0.01$). There was also marked testicular germ cell sloughing and degeneration. Adrenal gland hyperplasia and hypertrophy was also observed. The authors concluded that the observed changes, which occurred when the gari diet was consumed, were not entirely due to cyanide but to some other factor, probably intact linamarin (Kamalu, 1993).

Long-term carcinogenicity studies with cyanides

No long-term toxicity and carcinogenicity studies on HCN have been published.

Long-term carcinogenicity studies with cyanogenic glycosides

No data available.

Genotoxicity

Genotoxicity of cyanides
Two negative and one marginally positive bacterial mutagenicity studies have been reported. HCN was not mutagenic in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 with or without metabolic activation (De Flora, 1981, cited in EPA, 1990). Cyanide was negative in a recombinant-assay in Bacillus subtilis (Karube *et al*., 1981, cited in EPA, 1990). One study reported marginal mutagenic activity of HCN to *S. typhimurium strain* T 100 in the absence of metabolic activation but no mutagenic activity to strain TA 98 with or without metabolic activation (Kushi *et al*., 1983, cited in EPA, 1990)

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

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

Reproductive and developmental toxicity studies with cyanide

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

In rat
A short-term reproduction study (49 day study in adults and 28 day study in pups) was performed to evaluate the effects in pregnant rats of adding 500 mg KCN/kg to a cassava root flour-based diet prepared from a low-HCN cassava variety (21 mg HCN/kg feed). A control group received only the basal. The high dietary level of KCN did not have any marked effect on gestation and lactation performance of female rats. No carry-over effect of high cyanide-containing diet fed during gestation was observed on lactation performance. The high cyanide-containing diet, however, significantly reduced feed consumption and daily growth rate of the offspring when fed during the post-weaning period. Protein efficiency ratio was not only reduced by the cyanide diet during the post-weaning growth phase but there was a carry-over effect from gestation. Serum thiocyanate was significantly increased in lactating rats and their offspring during lactation and in the post-weaning growth phase of the pups. No apparent carry-over effect was noticed on this parameter. Rhodanese activity in liver and kidneys was unaffected by feeding the high cyanide diet during gestation, lactation, and or during post-weaning growth (Tewe and Maner, 1981b).

Special studies on the thyroid gland with cyanides

In rat
Six groups of 10 male weaning rats (average weight 43 g) were studied. One group was fed a 10 % casein diet containing added methionine, vitamin B_{12}, potassium iodine (positive control group). Two groups were fed with the above diet supplemented either with potassium cyanide (1500 mg/kg feed) or potassium thiocyanate (2240 mg/kg feed). Three groups received a 10% casein diet without iodine or vitamin B_{12} and with a lowered methionine level (negative control). The study was carried out for nearly one year. Compared to the control groups not receiving cyanide or thiocyanate, depression of body-weight was observed throughout the study period in the groups that were fed cyanide or thiocyanate, but there were no deaths or clinical sign of toxicity. Depression of both plasma thyroxin and thyroxin secretion rate, suggestive of depressed thyroid function, were evident in the treated groups at 4 months but less so after 1 year. At autopsy the animals were found to have enlarged thyroids and this may have been the mechanism of adaptation. Some differences in the histopathology of the spinal cord, notably the white matter, were also found between controls and cyanide-treated animals (Philbrick *et al*, 1979).

In pig
Performance and metabolic and pathological changes were evaluated in 48 growing pigs (Yorkshire) fed different levels of dietary protein (9 and 16 %), cyanide (0 and 500 mg KCN/kg feed), and iodine present at a level of 0.008% in a salt (0 and 0.36 mg iodine salt /kg feed, cation not specified) during 56 days. Protein deficiency reduced urinary iodine excretion and the concentrations of protein, protein bound iodine (PBI) and thiocyanate in serum. It also reduced liver rhodanese activity and caused a decrease in urinary thiocyanate excretion, which was not significant. Dietary cyanide increased urinary thiocyanate and iodine excretion and serum PBI. Pathological studies showed that cyanide treatment had no marked effect on the microanatomy of the tissues examined. Dietary iodine deficiency caused histological changes in the thyroid gland and bone suggesting a decline in metabolic activity. Iodine deficiency caused hyperplastic goitre in the experimental animals (Tewe and Maner, 1980).

Special studies on nervous system with cyanides

In pig
A special study on the behavioural effects of chronic sublethal dietary cyanide (KCN; 0, 0.4, 0.7 and 1.2 CN^-/kg bw/day) was carried out for 24 weeks in juvenile pigs (Pittman-Moore strain), mimicking the situation of intake of free CN in Liberia due to eating cassava based-foods. There were two clear behavioural trends: 1) increasing ambivalence and slower reacting response time to various stimuli, and 2) an energy conservation gradient influencing which specific behaviours would be modified in treated animals. Serum SCN^- was positively correlated with daily CN^- intakes. CN^- treatment diminished tri-iodothyronine and thyroxine levels but elevated fasting blood glucose values (Collier-Jackson, 1988).

In other animals
Neuronal lesions in several animal species have been produced by chronic cyanide intoxication either by injection of unbuffered alkaline cyanide salts or by inhalation of hydrogen 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 periventricular region. In some species, the earliest effects may be on the oligodendroglia and hence myelin lesions may precede neuronal damage. Bass, (l968) showed that in rats chronic cyanide intoxication produces myelin loss by its primary effect on glial cells followed by breakdown of myelin. Brierly *et al*. (1977) reported myelin damage and changes in the oligodendroglia in cyanide poisoning in rats. In these animal experiments relatively large doses of cyanide were given, often sufficient to cause partial anoxia. Therefore it was doubtful whether the neuropathological effects were due to anoxia or chronic cyanide intoxication and whether they have an obvious parallel to human exposure. It is noteworthy, however, that only in primates changes in optic nerves and tracts occur consistently (Ferraro, 1933; Hurst, 1940; Lessell, 1971).

In a study in which small weekly doses of cyanide were administered over several months to rats, neuronal degeneration and demyelination was reported (Smith *et al*., l963). Williams and Osuntokun (1969) found that the demyelination of peripheral nerves induced in rodents by cyanide injection bore a striking resemblance to the lesions found in biopsy specimen of peripheral nerves of Nigerian patients who suffer from tropical neuropathy (reviewed by Osuntokun, 1981).

To evaluate hydroperoxide generation as a potential mechanism of cyanide neurotoxicity, mice were given a single subcutaneous dose of 7 mg/kg bw KCN, and the level of lipid peroxidation (expressed as conjugated dienes) was measured in various organs. Brain tissue showed elevated conjugated diene levels at 15 and 30 minutes, but not 60 minutes after cyanide treatment; an increase was not found in the liver. Conjugated dienes in the kidney slowly increased to a peak, 1 hour after cyanide treatment.

In vitro studies showed an elevation of peroxidised lipids in mouse brain cortical slices following incubation with KCN. Sub-cellular fractionation of brains from mice treated with cyanide showed that lipid peroxidation increased in the microsomal fraction but not in the mitochondrial fraction. The authors suggested that hydroperoxide generation and the subsequent peroxidation of lipids may lead to changes in structure and function of certain membranes and contribute to the neurotoxic damage produced by cyanide (Ardelt. *et al*., 1994). Other *in vitro* studies suggest that cyanide appears to equilibrate rapidly across the plasma membrane and then slowly accumulates in the mitochondrial and membrane elements of the neuronal cell (Borowitz *et al*., 1994).)

Reproductive and developmental toxicity studies with cyanogenic glycosides

In rat
In a one generation reproduction study, albino female rats (10 rats/group) were fed *ad libitum* respectively: a normal laboratory diet (5% carbohydrate, 21% protein, 4% fat, 3.5% fibre, 0.098% calcium, 0.0025%

iron), a 50 % gari diet (Nigerian preparation of cassava), a raw cassava diet (content: 35.7% carbohydrate, 1.2% protein, 0.2% fat, 1.1% fibre, 0.0068% calcium, 0.0019% iron, 62% moisture, 0.36-2.5% hydrogen cyanide), a diet containing 5 g KCN/100 g laboratory diet and a diet containing 10 g KCN/100 g laboratory diet. After 2 weeks each group was mated with 5 adult males fed on normal diet. Pregnant rats from each group were maintained on their respective diets. After littering the newborn rats were studied for postnatal development. After 21 days F_1 rats were put for another 4 weeks on their respective diets.

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

In pig

General toxicity and reproductive effects were studied for cassava in combination with added cyanide. In a 110-day feeding experiment 18 pregnant Yorkshire gilts were allocated to three equal groups and fed fresh cassava (containing 40.2 mg HCN/kg fresh weight) supplemented with 0, 250 and 500 mg cyanide (KCN) per kg of fresh cassava offered.

Serum thiocyanate concentration was slightly but not significantly increased in the 500 mg KCN/kg group and serum protein bound iodine decreased during gestation in all groups. Foetal serum thiocyanate concentration was significantly ($p<0.05$) higher in the group fed 500 mg KCN/kg. A small increase in maternal thyroid weight with increasing levels of cyanide was observed. Pathological studies showed proliferation of glomerular cells of the kidneys in gilts of all groups and reduced activity of the thyroid gland in gilts fed 500 mg KCN/kg group. Cyanide fed during gestation did not affect performance during lactation. Serum thiocyanate and milk thiocyanate were significantly higher in lactating sows fed 500 mg KCN/ kg feed during pregnancy. Iodine concentration in the colostrum of the group fed 500 mg KCN/ kg feed was significantly higher than in the groups, which received lower levels of cyanide. No effects of cyanide were reported on the indices of reproduction performance (Tewe and Maner, 1981a).

In hamster

In a developmental toxicity study, pregnant hamsters received an oral dose of 70, 100, 120 or 140 mg linamarin/kg bw during the early primitive streak stage of gestation (day 8 of gestation). The hamsters were killed on the morning of day 15 of pregnancy. Foetuses were removed by caesarian section and the numbers of resorption sites, dead foetuses, and living foetuses were recorded. Living foetuses were examined for gross external malformations and by means of histopathological methods for internal malformations. A dose of 120 or 140 mg linamarin/kg bw was associated with an increased incidence of vertebral and rib anomalies as well as the production of encephaloceles in the offspring. These larger doses of linamarin also resulted in obvious maternal toxicity (dyspnea, hyperpnea, ataxia, tremors and hypothermia). Two animals dosed with 140 mg and one animal dosed 120 mg/kg bw died. In surviving animals the signs of poisoning were greatly reduced or gone within 3 h after treatment. Linamarin treatment had no effect on foetal body weight, ossification of skeletons, embryonic mortality, or litter size. Although ingestion of the cyanogenic glycoside was associated with a significant teratogenic response, the effects occurred only at the doses that produced signs of maternal intoxication (Frakes *et al*., 1985).

In another developmental toxicity study, groups of pregnant hamsters (8 dams/group) were fed diets consisting of cassava meal:laboratory chow (80:20) during days 3-14 of gestation. One low cyanide (sweet) cassava meal and one high cyanide (bitter) cassava meal were studied. One additional group was fed a diet which resembled cassava in nutritional value, but which lacked cyanogenic glycosides. Thiocyanate concentrations in the urine and blood of dams fed cassava diets increased significantly. Increased tissue thiocyanate concentrations were observed in foetuses recovered from cassava-fed dams. Cassava-fed dams gained significantly less weight than did control animals and their offspring showed evidence of foetotoxicity. Reduced foetal body weight and reduced ossification of sacrocaudal vertebrae, metatarsals and sternebrae were associated with cassava diets. High cyanide cassava diets were also associated with a significant increase in the numbers of runts compared to litters from dams fed either low protein or laboratory stock diets (Frakes *et al*., 1986a).

Genotoxicity of cyanogenic glycosides

No data available

Human data

Acute toxicity of cyanides in humans

The acute lethal oral dose of HCN for human beings is reported to be 0.5-3.5 mg/kg bw (0.48- 3.37 mg CN^- /kg bw) corresponding to 1.0-7.0 mg/kg bw of KCN (Montgomery, 1969; Gosselin *et al*., 1976; Geitler & Baine, 1983). The clinical signs are well described and include headache, dizziness, mental confusion, stupor, cyanosis with twitching and convulsions, followed by terminal coma (Conn, 1979b).

Acute toxicity of cyanogenic glycosides

One to 10 g of amygdalin have been given parenterally in humans without apparent acute toxic effects. This indirectly suggests that there is no significant metabolism of the intact injected glycoside (Morrone, 1962; CMA, 1953).

With oral dosing of amygdalin, a toxic potential is manifest. β-Glucosidase is present in the gastrointestinal lumen, a contribution of intestinal microflora. According to Eyerly (1976; cited in Dorr and Paxinos, 1978) laetrile (amygdalin) given orally could be 40 times more toxic than given parenterally. This is probably due to the release of free HCN by the β-glucosidase enzyme present in the gut.

If it is assumed that about 100-2000 mg cyanide is the lethal dose for man, as much as 10-20 kg of cassava meal (‘lafun’; 10-20 mg cyanide/kg) will have to be consumed at a single sitting to produce toxicity (Oke, 1980).

Well-nourished individuals have ingested 1000 mg (equivalent to 59 mg HCN) or more of pure amygdalin every day without any evidence of “side effects” (Oke, 1979).

In a case study, an 11-month-old girl was reported to accidentally have ingested 1-5 amygdalin tablets (500 mg, equivalent to 29.5 mg HCN). The patient became listless within half an hour after ingestion and vomited. Breathing became irregular and her state of consciousness became altered. An hour after ingestion she was in a shock and died approximately 72 hours following ingestion in spite of hospital treatment (Humbert *et al.*, 1977).

In a case study, a 17-year-old girl suffering from cancer made a practice of taking, instead of radiotherapy, four ampoules of laetrile (3 g amygdalin, equivalent to 177 mg HCN) intravenously. One day she swallowed orally the content of 3.5 ampoules of laetrile. Shortly after ingestion, a severe headache and

dizziness developed, and she collapsed. Laboured breathing developed, her pupils became dilated, and she became comatose. All symptoms occurred within 8-10 minutes after ingestion. She died 24 hours after ingestion. (Sadoff *et al*., 1978).

In Anatolia (Turkey) 9 cases of cyanide intoxication of children due to the ingestion of wild apricot seeds (217 mg HCN/100 g) were reported. The victims had probably eaten more than 10 seeds. It is not indicated whether death occurred. Quantitative figures on cyanogenic glycoside or cyanide intake are not given (Sayre and Kaymakcalavu, 1964).

In a case-study a 67-year-old woman collapsed after ingestion of a slurry of 12 bitter almonds grounded up and mixed with water. She recovered after treatment in the hospital. The average cyanide content was 6.2 mg HCN/bitter almond (Shragg *et al*., 1982).

In a reported case, ingestion of 30 g of bitter almonds caused severe intoxication in a 20-year-old man. The authors state that one bitter almond contains the equivalent of 1 mg HCN and calculate that the consumption of 60 bitter almonds may be lethal for an adult and 5-10 for a child (Askar and Morad, 1983).

Long-term toxicity of cyanogenic glycosides and cyanides

Several disorders observed in cassava-eating populations have been attributed to the effects of chronic exposure to dietary cyanide. These include malnutrition, diabetes, congenital malformations, neurological disorders and myelopathy (Baumeister, 1975; Davidson, 1979). Goitre is thought to have occurred when cyanogenic glycosides were present at a level of 10-50 mg/kg in food.

A study to evaluate the possible association of high cyanide and low sulphur intake in cassava-induced spastic paraparesis was performed. The north-eastern part of Mozambique suffered in 1981 a severe drought: the crop to survive was the most toxic variety of cassava and, due to lack of food during the harvest period, the roots were eaten after only a few days of sun drying. A field survey revealed 1102 cases of spastic paraparesis. In 1982 urine was collected from 30 apparently healthy children (age 8.1 years). As reference 17 Swedish children (age 8.6 years) were used.

In second stage, urine was sampled in 1983 (when the nutritional situation was improved but still unsatisfactory) from 31 children (9.0 years) in the same village (the authors do not indicate if this were the same children as in the 1982 study) and 30 schoolchildren (8.1 years) in a nearby district where no cases of paraparesis were seen in 1981 and from 28 children (7.1 years) of the city who ate virtually no cassava. The children from the village had increased thiocyanate and decreased inorganic sulphate excretion, indicating high cyanide and low sulphur-containing amino acid intake. Children from a neighbouring cassava eating area, where no cases of spastic paraparesis had occurred, had lower thiocyanate excretion but higher organic sulphate excretion. These results support the hypothesis that the epidemic was due to the combined effects of high dietary cyanide exposure and sulphur deficiency (Cliff *et al*, 1985).

In Nigeria, several studies, including epidemiological studies, were carried out on the role of chronic cyanide intoxication by the consumption of cassava diet in the etiology of tropical (ataxia) neuropathy (TAN). Chronic non-lethal exposure to cyanide was assessed by measuring plasma thiocyanate and cyanide levels, 24-hour urinary thiocyanate, quantitative and qualitative estimations of sulphur-containing amino acids, various components of vitamin B_{12} (cyanocobalamin, hydroxycobalamin, deoxyadenosylcobalamin, methylcobalamin) in plasma and liver and rhodanese activity in liver. As detailed in over 400 Nigerian patients the essential neurological components of the disease are myelopathy, bilateral optic atrophy, bilateral perceptive deafness and polyneuropathy. The initial and most common symptoms consist of various forms of paraesthesiae and dysaesthesia usually starting in the distal part of the lower limbs. The next most common finding is blurring or loss of vision. Other common symptoms in order of frequency are ataxia, tinnitus, deafness, weakness and thinning of the legs. In about a third of the patients,

stomatoglossitis is present, whereas motor neurone disease, Parkinson's disease, cerebellar degeneration, psychosis and dementia have been associated with the disease. TAN affects males and females equally and all age groups but occurs only rarely in children under 10 years. Patients usually give a history of almost total dependence on a monotonous diet of cassava derivatives, and occasional dietary supplements include yam, maize, rice, vegetables and animal protein. Analysis of the relationship showed no evidence of a genetically determined predisposition. The families were usually poor and members lived communally. Clinical evidence of malnutrition was frequently absent. The significant higher cyanide and thiocyanate plasma levels and higher excretion of thiocyanate in the patients than in the controls indicated the occurrence of cyanide intoxication in Nigerian patients. Hepatic rhodanese activity was not different from those of controls and histology of liver biopsy specimens showed no abnormality. Total plasma vitamin B_{12} levels are normal or high in patients and healthy Nigerians but plasma concentration of cyanocobalamin was highly significantly raised in patients. A small proportion of cyanocobalamin was found in the liver of patients. Methylmalonic acid excretion was normal in patients, indicating that there was physiological adequacy at tissue or cellular level of vitamin B_{12} in these patients (Osuntokun, 1981).

The frequency of cassava consumption was investigated among three groups of people: students from both traditional and non-traditional cassava-consuming environments and cassava processors. Of these, 64% of the traditional consumers, 38% of the non-traditional consumers and 44% of the cassava processors consumed cassava products at least once a day, while 4, 35, and 28% of the groups respectively, were moderate cassava consumers (4-6 times a week). The serum thiocyanate levels of the processors were significantly higher (0. 5 mg/dl) than those of the other groups (0.38 mg/dl), but there was no significant difference in the urinary thiocyanate level of the three groups. Analysis of cassava and its intermediate and final products for free cyanide, acetone cyanohydrin, and intact glycosides during the production of cassava products revealed that while the finished products might be safe for human consumption, the workers were probably exposed at different stages of processing to a non-dietary source of cyanide (Adawusi *et al*., 1994).

Konzo is a distinct form of tropical myelopathy (spastic paraparesis), the disease having been attributed to dietary cyanide exposure from insufficiently processed cassava. A Konzo-affected population in former Zaire was investigated and compared to an unaffected population; the cyanogen content of cassava flour was measured, urinary thiocyanate was determined as an indicator of cyanide intake and blood cyanide concentrations in cases and controls were compared. The affected population consumed flour made from short-soaked cassava roots and thus were exposed to high levels of dietary cyanide (mean urinary thiocyanate in 31 children was 757 µmol/l) compared with the unaffected population (mean urinary thiocyanate in 46 children was 50 µmol/l) who ate cassava, which had been soaked for three days before consumption. Three Konzo patients, but only 2 of the 23 controls from the unaffected population, had blood cyanide concentrations above 4 µmol/l, although serum thiocyanate concentrations were similar. The affected population had a daily intake of cassava flour of above 0.5 kg per adult equivalent to an intake of 0.19-0.37 mg CN/kg bw/day (Tylleskar *et al*., 1992).

The thiocyanate load resulting from the endogenous conversion of cyanide has been reported as an etiological factor in endemic goitre and cretinism among cassava eaters (Osuntokun, 1980). The high thiocyanate excretion found in cassava-eating children from the rural area affected by an epidemic of spastic paraparesis and degenerative neuropathy indicates that cyanide intake was high during the cassava harvest the first year after the epidemic was reported; it was slightly lower the following year. The results also suggest a high dietary cyanide intake in cassava-eating children from the neighbouring semi-urban area not affected by the epidemic and, as expected, a very low cyanide intake in urban children (Vennesland *et al*., 1982).

The incidence of tropical amblyopia (defective vision) and ataxic neuropathy rose sharply among people who lived on uncooked and unprocessed cassava roots during the Nigerian 1969 civil war. The deficiency of sulphur amino acids in cassava root proteins may result in failure to convert ingested cyanide to the less

toxic thiocyanate and it may, thus, be the reason for the ophthalmological and neurological complications of chronic cyanide toxicity. In studies with Nigerian patients and in a study with West-Indian people with optic neuropathy there was also an increase in plasma cyanocobalamin and urinary thiocyanate levels, products of cyanide detoxification, suggesting that dietary cyanide may be involved in the pathogenesis of tropical amblyopia (Roslin, 1986; Osungawa, 1989; Freeman, 1988; Osuntokun, 1971).

Urinary excretion of sulphur compounds was studied in children from a population in Mozambique that had been affected by an epidemic of spastic paraparesis attributed to cyanide exposure from cassava. High thiocyanate and low inorganic sulphate excretion were found in the children, indicating a high intake of cyanide and a low intake of sulphur-containing amino acids compared to Swedish controls. In children from a neighbouring cassava-eating area where no cases of spastic paraparesis had occurred, excretion of thiocyanate was lower and excretion of inorganic sulphate higher than in the affected area. These results support the hypothesis that the epidemic was due to the combined effects of high dietary cyanide exposure and sulphur deficiency (Cliff, *et al*., 1985). This is further supported by a study in Tanzania where average levels of urinary thiocyanate were found to be 490 ± 48 µmol/l in a village affected by Konzo and 300 ± 39 µmol/l in an unaffected neighbouring village. Urinary sulphate levels were 3802 ± 369 µmol/l in the affected village and 7038 ± 855 µmol/l in the unaffected village (Mlingi *et al*., 1993). Other workers, however, consider that zinc deficiency may be an additional factor in the development of Konzo (reviewed by Kamalu, 1995).

A case control study conducted in Konzo-affected and unaffected villages in former Zaire demonstrated that while cyanide, as measured by urinary thiocyanate, had an etiological role in the development of the condition, urinary linamarin levels were more closely associated with it. The authors suggest that linamarin itself may have a specific neurotoxic effect. Levels of urinary inorganic sulphate were low in all groups. However, the differences between urinary thiocyanate and linamarin levels in affected patients and unaffected members of the same household are low (compared to the differences between levels in subjects from affected and unaffected villages), so the study does not explain why some individuals in the same household are more prone to the development of this condition (Tylleskar *et al*., 1992; Banea-Mayambu *et al*., 1997).

Other authors argue that the slow developing tropical neuropathy associated with consumption of a cassava based diet is not due to cyanogenic glycoside but to the presence of scopoletin (a coumarin), a potent hypotensive and non-specific spasmolytic agent present in cassava broth as a result of contamination with *Aspergillus flavus* (Obidoa, and Obasi, 1991)

DISCUSSION

The quantitative data on oral acute toxicity of HCN are limited to studies in dog and rat in which the LD_{50} was found to be respectively 5.3 mg KCN/kg bw and 10-15 mg KCN/kg bw, equivalent to 2.13 and 4.02-6.03 mg CN^-/kg bw. The lowest published lethal dose to humans was 0.56 mg HCN/kg bw, indicating that humans are more sensitive to acute effects of HCN. No safe intake level with respect to the acute toxicity of HCN has been established. The peak-plasma levels of HCN determine the acute toxicity. In case of intake of cyanogenic glycosides HCN is slowly and incompletely released by hydrolysis of gut microflora and then absorbed and the acute toxicity for HCN will be lower. The lethal oral dose of the cyanogenic glycoside linamarin in the rat was determined to be 450 mg/kg bw.

In the NTP 13 weeks study with rats, administration of NaCN at doses up to 24 NaCN/kg bw/day in drinking water resulted in no significant adverse effects on body weights, organ weights, histopathology, or clinical pathology parameters. No evidence of neurological or thyroidal gland damage was seen. The absorption of administered cyanide was confirmed by increases in urinary thiocyanate excretion. The reported reduction in sperm motility was minor and not dose related and therefore of doubtful significance.

The only significant effect in rats was the reduction of sperm heads per testis at the highest dose level.

In male mice the only effect with toxicological significance was slightly reduced cauda epididymal weight and reduced sperm motility at the highest dose level of 46 mg NaCN/kg bw/day. Based on these effects, for mice a NOAEL of 16 mg NaCN/kg bw/day can be derived, corresponding to 8.5 mg CN^-/kg bw/day.

A similar study was conducted in mice.

In a limited study in which dogs were fed a rice-based diet supplemented with NaCN or cassava (gari) for 14 weeks, both resulting in an exposure of 1.08 mg/kg bw/day, significant effects were observed on kidney, adrenal and testis and spermatogenesis. Comparison of this dose with the NOAELS for rats and mice suggests that dogs are more sensitive to cyanide than rats or mice.

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

Experimental data on chronic toxicity and carcinogenicity are not available.

Overall, the mutagenicity tests conducted with HCN and cyanides at gene and/or chromosome level did not reveal a genotoxic potential.

The Panel considered several epidemiological studies in cassava-eating populations, which established an association between cyanide exposure and spastic paraparesis, amblyobia ataxia or tropical ataxia neuropathy (TAN) and possibly goitre. However, the data were not considered adequate to establish a numerical NOAEL for chronic exposure in humans, because they were highly confounded by other nutritional and environmental factors. Adequate long-term toxicity studies in animals fed a diet containing HCN or cyanogenic glycosides to derive a NOAEL are also lacking. Therefore, no overall NOAEL is available on which to base a numerical TDI.

In 1993, the JECFA concluded: “Because of a lack of quantitative toxicological and epidemiological information, a safe level of intake of cyanogenic glycosides could not be established.”. However, the JECFA also concluded that at the Codex standard for cassava flour up to 10 mg HCN/kg cassava flour (CAC, 1991) is not associated with acute toxicity (WHO, 1993).

Cassava flour is used as a staple food mainly outside Europe; a consumption of 200 g/person would lead to an estimated intake level of 30 µg HCN/kg bw for a 60 kg adult. In accordance with the JECFA view such an intake would not be associated with acute toxicity. The highest level of HCN found in retail marzipan paste is 20 mg HCN/kg. Assuming on one sitting a person of 60kg consumes 100 g marzipan containing such a level, that intake would be equivalent to 2 mg HCN or to 0.03 mg/kg bw.

Limited data from the UK show that the average and high (97.5^{th} percentile) daily intake of HCN from its use in flavours or flavour ingredients were 46 and 214 µg/person, which correspond to approximately 0.8 and 3.6 µg/kg bw/day respectively.
Data of from a Norwegian dietary survey show that the average and high (97.5^{th} percentile) daily intake of HCN among consumers amounts to respectively 95 and 372 µg/person or 1.4 and 5.4 µg/kg bw/day.

CONCLUSION AND RECOMMENDATION

The Panel concluded that the current exposure to cyanide from flavouring ingredients (at the 97.5 percentile 3.6 µg/kg bw/day) is unlikely to give rise to acute toxicity. The overall data were not considered adequate to

establish a numerical NOAEL or TDI in humans for chronic exposure. In view of the lack of adequate data on chronic toxicity the Panel supports the continued application of limits for the presence of HCN in foods and beverages.

DOCUMENTATION PROVIDED TO EFSA

None

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