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Acute health risks related to the presence of cyanogenic glycosides in raw apricot kernels and products derived from raw apricot kernels

EFSA Panel on Contaminants in the Food Chain (CONTAM)

Abstract

Amygdalin is the major cyanogenic glycoside present in apricot kernels and is degraded to cyanide by chewing or grinding. Cyanide is of high acute toxicity in humans. The lethal dose is reported to be 0.5–3.5 mg/kg body weight (bw). An acute reference dose (ARfD) of 20 μg/kg bw was derived from an exposure of 0.105 mg/kg bw associated with a non-toxic blood cyanide level of 20 micro mol (μM), and applying an uncertainty factor of 1.5 to account for toxicokinetic and of 3.16 to account for toxicodynamic inter-individual differences. In the absence of consumption data and thus using highest intakes of kernels promoted (10 and 60 kernels/day for the general population and cancer patients, respectively), exposures exceeded the ARfD 17–413 and 3–71 times in toddlers and adults, respectively. The estimated maximum quantity of apricot kernels (or raw apricot material) that can be consumed without exceeding the ARfD is 0.06 and 0.37 g in toddlers and adults, respectively. Thus the ARfD would be exceeded already by consumption of one small kernel in toddlers, while adults could consume three small kernels. However, consumption of less than half of a large kernel could already exceed the ARfD in adults.

Keywords: cyanogenic glycosides, cyanide, apricot kernels, acute reference dose

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Summary

Following a request from the European Commission, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) assessed acute risks to human health related to the presence of cyanogenic glycosides (CNGs) in raw apricot kernels or products derived thereof.

Risk assessments from EFSA and other international and national scientific bodies on CNGs together with the publications referenced therein have been used as a starting point for the identification and characterisation of acute health hazards of cyanide. In addition, a systematic literature search has been carried out to obtain up-to-date and comprehensive information on hazards of CNGs and their occurrence in apricot kernels and products thereof. No consumption data on apricot kernels were available in the EFSA database. For hazard and exposure assessment the general principles for risk assessment were followed.

Amygdalin is the major CNG present in apricot kernels. In intact apricot kernels, amygdalin and its catabolic enzymes are stored in separate compartments and are brought into contact by physical processes such as grinding or chewing thereby releasing hydrocyanic acid (HCN). Complete degradation of 1 g of amygdalin releases 59 mg HCN. Because of its weak acidity, in aqueous biological fluids HCN always exists as a mixture of non-dissociated acid (HCN) and its dissociated form (as cyanide anion or CN^-), the proportion of either depending on the pH in the fluid. The term cyanide, as used in the present assessment, thus represents dissociated and non-dissociated forms. Amygdalin is also metabolised to cyanide in the intestinal tract by the gut microbiota.

Cyanide is readily absorbed reaching maximum blood levels within minutes and is distributed to all organs. Its half-life in blood is usually less than 1 h. There are no pronounced species differences in the toxicokinetics of cyanide. The primary mode of action by which cyanide exerts acute toxicity is the inhibition of oxidative phosphorylation, in particular of cytochrome oxidase a3. Heart and brain are the most sensitive organs because they require continuous supply of ATP generated by oxidative phosphorylation.

Acute oral median lethal dose (LD_{50}) values for cyanide in laboratory animals range from 2.13 to 6 mg/kg body weight (bw). Lethal levels of cyanide lead to dyspnoea, irregular and gasping breathing, ataxia, tremor, spasms, loss of consciousness, convulsions and eventually asphyxiation. Short-term dietary exposure to cyanide leads to histopathological changes and alterations in organ weights. For cyanide a no observed adverse effect level (NOAEL) of 0.36 mg cyanide/kg bw per day [lowest observed adverse effect level (LOAEL of 1.2 mg cyanide/kg bw per day)] was reported in rats. There are indications that cyanide can act as a teratogen at higher doses.

In humans, the lethal oral dose of HCN is reported to be 0.5–3.5 mg/kg bw. A level of 0.5 mg/L (approximately 20 micro mol, μM) of cyanide in blood is cited in the literature as a toxicity threshold in humans. A series of poisoning cases are reported from ingestion of preparations containing amygdalin and bitter apricot kernels. In adults, 20 or more kernels were reported to be toxic while the corresponding number in children was five.

The animal data available did not provide a suitable basis for acute human health hazard assessment. The CONTAM Panel concluded that the blood cyanide concentration of 20 μM, which is reported to be a threshold for toxicity should be used as the basis for deriving the acute reference dose (ARfD), extrapolating to an external dose of cyanide. In a bioavailability study, a group of 12 healthy adult volunteers consumed CNGs corresponding to 6.8 mg cyanide from a number of different sources including bitter apricot kernels. A cyanide exposure of 0.105 mg/kg bw was estimated to result in a mean peak blood level of 20 μM in the females in the study. The CONTAM Panel concluded that the dose of 0.105 mg/kg bw, resulting in a blood concentration of about 20 μM in the females, should be used as the basis for deriving the ARfD. Taking into account that it is assumed that CNGs are completely broken down to cyanide and that the cyanide is rapidly absorbed and 100% bioavailable, it is highly unlikely that individual variability could lead to appreciably higher peak blood levels. Because of the very short time to peak blood level, individual differences in metabolism (e.g. due to reduced activity of rhodanese) could delay elimination, but are unlikely to have an impact on the peak level. The variations in peak blood levels seen in the study from Abraham et al. (2016) were small. Women have a smaller distribution volume of blood than men and children have a larger blood volume (per kg/bw) than adults. Therefore, the CONTAM Panel concluded that a default factor of 3.16 was not required and that a factor of 1.5 was sufficient to cover any additional variability in toxicokinetics. The CONTAM Panel noted the lack of information on whether potentially sensitive individuals (e.g. children) were included in the database underpinning the assumption that a blood cyanide level of 20 μM is a toxicity threshold, and that the bioavailability study was conducted in a small number of healthy

volunteers. The CONTAM Panel concluded that a toxicodynamic subfactor should be applied, and in the absence of cyanide-specific data on individual sensitivity, adopted the default subfactor of 3.16. Dividing the NOAEL of 0.105 mg/kg bw by the factors of 1.5 and 3.16, with rounding to a single significant figure, an ARfD for cyanide of 0.02 mg/kg bw (20 μg/kg bw) was established for use in assessing the risks associated with the presence of CNGs in apricot kernels.

On the basis of information provided in the literature, an average kernel weight of 0.5 g and cyanide concentrations ranging from 0.5 to 3.8 mg/g kernel were assumed for exposure assessment and risk characterisation. The available information on amygdalin/cyanide content was not sufficient to discriminate between 'bitter' and 'sweet' apricot kernels. Due to lack of data it was not possible to separately assess products containing raw apricot kernels or processed (e.g. ground, or without skin) raw apricot kernels.

In the absence of consumption data, the highest number of kernels promoted by apricot kernel vendors for the general population and cancer patients (10 and 60 kernels respectively) was used for exposure assessment together with the highest and lowest mean values for cyanide concentrations in apricot kernels described as 'bitter'. For the general population, resulting exposures were 333 and 57 μg/kg bw assuming low cyanide content and 1,375 and 236 μg/kg bw assuming high cyanide content, for toddlers and adults, respectively. For cancer patients, the corresponding numbers were 2,000 and 343 μg/kg bw and 8,250 and 1,414 μg/kg bw. In all scenarios, the ARfD of 20 μg/kg bw was substantially exceeded.

Using the highest cyanide concentration in apricot kernels reported in the literature, the estimated maximum quantity of apricot kernels (or raw apricot kernel material) that could be consumed by a toddler without exceeding the ARfD was 0.06 g while an adult could eat up to 0.37 g. It is not possible for consumers to measure such small quantities in the home. For a toddler, the ARfD would be exceeded by consuming less than one small kernel. For adults, three very small kernels could be consumed, but consumption of less than half of a large kernel could already exceed the ARfD.

The overall uncertainty incurred with the present assessment is considered as high. It is more likely to overestimate than to underestimate the risk.

Information is needed on whether there is an objective distinction between 'sweet' and 'bitter' apricot kernels, and if so whether the cyanide contents differ. Data are also needed on the occurrence of cyanide in whole raw apricot kernels and products derived from them. Furthermore, more information is required on the impact of removing the skin and other forms of processing on the cyanide content, as well as on consumption of products prepared from raw apricot kernels.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

There has been a number of poisoning incidents in both Australia and New Zealand following consumption of raw apricot kernels that contained high levels of hydrocyanic acid (HCN). This poses an ongoing risk that needs to be managed to avoid future poisoning incidents.

In order to manage the potential risks of consumption of raw apricot kernels, Australia and New-Zealand have prepared a draft food regulatory measure to prohibit the sale of raw apricot kernels both with and without skin. This prohibition would also apply to any substance derived from raw apricot kernels (ground, milled, cracked, chopped) with an exemption for apricots containing raw apricot kernels, alcoholic beverages, oil, flavourings, stone fruit juices, marzipan, cakes, biscuits and confectionery.

The presence of HCN is regulated by Regulation (EC) No 1334/2008[1] in flavourings and food ingredients with flavouring properties, in certain compound food as consumed to which flavourings and/or food ingredients with flavouring ingredients with flavouring properties have been added. A maximum level of HCN of 50 mg/kg has been established in nougat, marzipan or its substitutes or similar products, of 5 mg/kg in canned stoned fruits and of 35 mg/kg in alcoholic beverages.

Regulation (EC) No 110/2008[2] establishes a maximum content of HCN of 7 g/hL of 100% volume alcohol in fruit marc spirit and stone fruit spirits.

There are no European Union (EU) provisions as regards the presence of HCN in (raw) apricot kernels. The presence of very high levels of HCN in apricot kernels (between 333 and 2,545 mg/kg) has been reported several times to the Rapid Alert System for Food and Feed (RASFF).

In the Expert Committee 'Agricultural contaminants' on 15 January 2015, the presence of HCN in apricot kernels was acknowledged to be a potential acute health risk for consumers in the EU and therefore restrictive measures at EU level might also be appropriate. The need for restrictive measures in the frame of Regulation (EEC) 315/93[3] will be decided taking into account the outcome of a risk assessment performed by the European Food Safety Authority (EFSA) on the acute health risk from the presence of HCN in apricot kernels and derived products. The Standing Committee on Plants, Animals, Food and Feed has been informed thereof at its meeting on 11 February 2015.[4]

1.1.2. Terms of Reference

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002, the Commission asks EFSA for a scientific opinion on the acute human health risks related to the presence of HCN in apricot kernels and products derived from apricot kernels (ground, milled, cracked, chopped).

1.2. Interpretation of the Terms of Reference

The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) noted that free HCN is not present in apricot kernels and that any risks are related to the release of HCN from cyanogenic glycosides (CNGs) present in apricot kernels. Following the terms of reference provided by the European Commission, only apricot kernels and products derived thereof that were not heat processed or treated were considered in the assessment. Because of its weak acidity, HCN always exists as a mixture of non-dissociated acid (HCN) and its dissociated form (cyanide ions, CN^-) in aqueous biological fluids, the proportion of either form in the dissociation equilibrium depending on the pH of

[1] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 16011, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

[2] Regulation (EC) No 110/2008 of the European Parliament and of the Council of 15 January 2008 on the definition, description, presentation, labelling and the protection of geographical indications of spirit drinks and repealing Council Regulation (EEC) No 1576/89. OJ L 39, 13.2.2008, p. 16–54.

[3] Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1–3.

[4] Summary report of the Standing Committee on Plants, Animals, Food and Feed, held in Brussels on 11 February 2015 (Section Toxicological Safety of the Food Chain), agenda item A.06. Report available at: http://ec.europa.eu/food/committees/regulatory/scfcah/toxic/docs/sum_20150211_en.pdf

the fluid. Therefore, the term 'cyanide' will be used throughout this opinion to inclusively represent the inorganic forms of cyanide, i.e. the undissociated HCN and the dissociated CN^-.

The CONTAM Panel also noted that apricot kernels are sometimes described as 'bitter' or 'sweet', and decided to determine whether a distinction could be made between these.

1.3. Additional information

1.3.1. Chemistry

Hydrocyanic acid (hydrogen cyanide or HCN) does not occur in apricot kernels as free compound but 'hidden' in the glycosides amygdalin and prunasin. Amygdalin and prunasin are members of a large class of natural products called CNGs because they are enzymatically degraded when ingested and release HCN, which exists in a dissociation equilibrium with CN^- in aqueous biological fluids. The mixture of non-dissociated HCN and cyanide ions is termed 'cyanide' (see Section 1.2) which is the cause of the toxic effects of apricot kernels. Before discussing the chemistry of cyanide, the chemistry of amygdalin, as well as its biosynthesis and degradation will be described briefly.

Amygdalin is the trivial name for (R)-α-[(6-O-β-D-glucopyranosyl-b-Dglucopyranosyl)-oxy]-(phenyl)acetonitrile, also named D-(-)-mandelonitrile-β-D-gentiobioside (Figure 1, left formula). It has the Chemical Abstracts Service (CAS) number 29883-15-6, the molecular formula $C_{20}H_{27}NO_{11}$, and a molecular mass of 457.4 g mol^{-1}. Amygdalin is a colourless substance with a melting point of 213°C. It is well soluble in ethanol, moderately soluble in water (83 g L^{-1} at 25°C) and non-soluble in non-polar solvents such as benzene or chloroform. The name vitamin B17 occasionally used for amygdalin is erroneous, because it is not a vitamin. Amygdalin is also sometimes referred to as laetrile. This designation is also wrong, as the real laetrile is (L-(-)-mandelonitrile-β-D-glucuronide, CAS number 1332-94-1), a semisynthetic cyanogenic glucuronide promoted as an alternative anticancer agent with a chemical structure different from that of amygdalin (Figure 1, right formula).

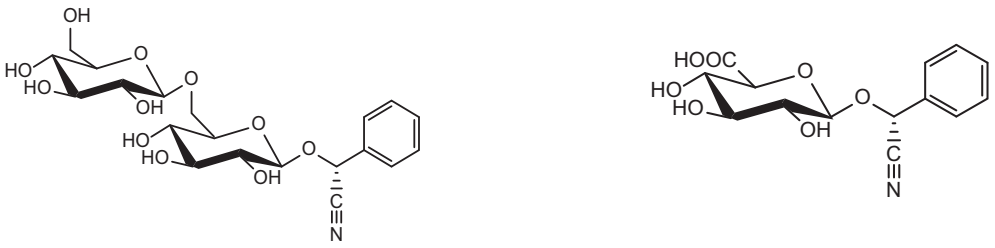


Figure 1: Chemical structures of amygdalin and laetrile

The biosynthesis of amygdalin in plants follows the general scheme for CNGs i.e. consecutive hydroxylation of an amino acid by cytochrome P450 (CYP) enzymes to an oxime and subsequently α-hydroxynitrile, followed by glycosylation of the latter (Ganjewala et al., 2010; Gleadow and Møller, 2014). In the case of amygdalin (Figure 2), the amino acid phenylalanine is hydroxylated by a CYP79 enzyme to phenylacetaldoxime, which is further hydroxylated by a CYP71 enzyme to mandelonitrile. Subsequent attachment of one glucose molecule to the α-hydroxyl group of mandelonitrile, catalysed by a uridine diphosphate glucose-glucosyl transferase (UGT) leads to prunasin (D-(-)-mandelonitrile-β-D-glucoside, CAS number 99-18-3, 295.3 g mol^{-1}), which is finally converted to amygdalin by attaching another glucose molecule to the 6'-hydroxyl group, thereby forming the diglucoside gentiobiose (Yamaguchi et al., 2014).

Neoamygdalin (CAS number 29883-16-7) is the S stereoisomer of amygdalin. It is not formed during the biosynthesis of amygdalin in the apricot kernel but can be generated from amygdalin via isomerisation of the stereogenic mandelonitrile carbon in aqueous solution under mild basic conditions (Krieble, 1912; Wahab et al., 2015). Amygdalin (R-amygdalin) and neoamygdalin (S-amygdalin) are diastereomers and not enantiomers because the configurations of the 10 stereogenic centres of their gentiobiose moieties do not change (Wahab et al., 2015). As diastereomers, amygdalin and neoamygdalin can be separated by chromatography on achiral stationary phases (see Section 1.3.2).

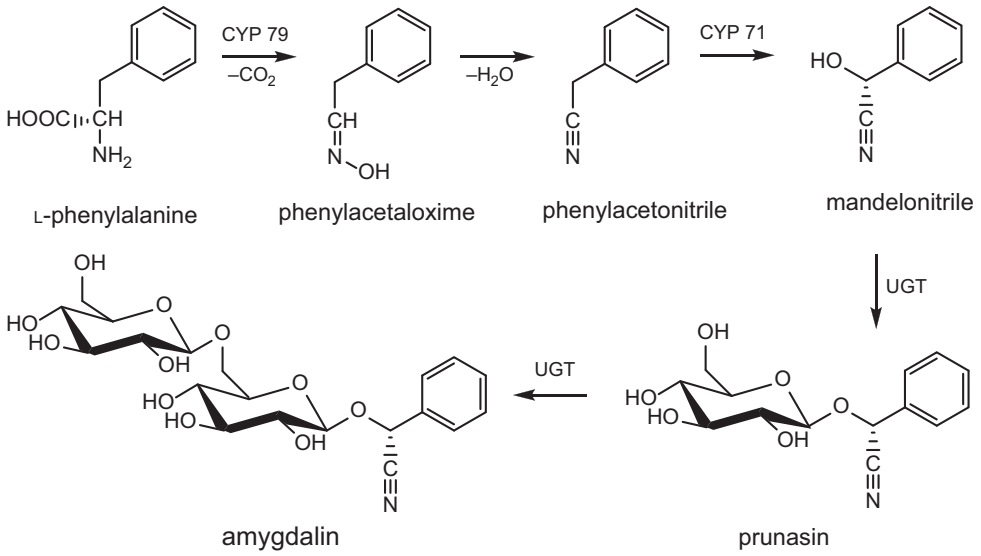
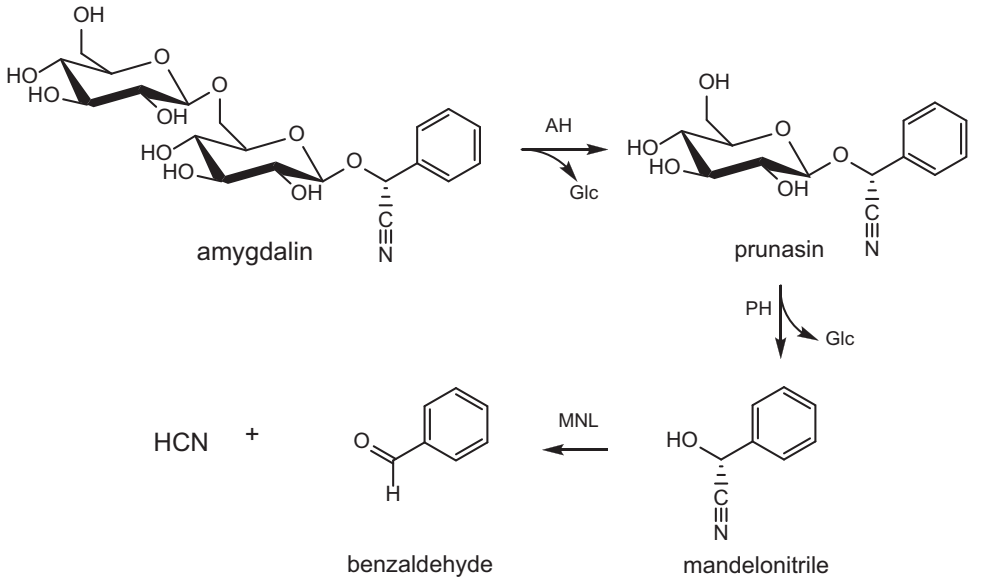


Figure 2: Biosynthesis of amygdalin

In contrast to CNGs, which are chemically stable and stored in plants, the biosynthetic intermediate mandelonitrile (which is chemically a cyanohydrin or α-hydroxynitrile) is labile and able to spontaneously dissociate into benzaldehyde and HCN would it not be stabilised by glycosylation. Thus, if the glycosidic bond with mandelonitrile is hydrolysed, a process known as cyanogenesis is initiated (Figure 3). The first step in the degradation of amygdalin is mediated by the β-glucosidase amygdalin hydrolase [Enzyme Commission Number (EC) 3.2.1.117] to yield prunasin and one glucose molecule. Prunasin is then hydrolysed by another β-glucosidase named prunasin hydrolase (EC 3.2.1.21) to mandelonitrile and glucose. The conversion of mandelonitrile to benzaldehyde and HCN proceeds spontaneously at neutral or alkaline pH, but is much faster in the presence of the enzyme mandelonitrile lyase (EC 4.1.2.10; Swain and Poulton, 1994). Complete hydrolysis of 1 g of amygdalin generates 59 mg of HCN (FAO/WHO, 2012).

So far, no evidence for a 'direct' hydrolysis of amygdalin to mandelonitrile and gentiobiose has been reported (Gleadow and Møller, 2014).



AH: amygdalin hydrolase; PH: prunasin hydrolase; MNL: mandelonitrile lyase; Glc: glucose.

Figure 3: Formation of hydrocyanic acid from amygdalin and prunasin

The process of cyanogenesis is sometimes also referred to as the 'cyanide bomb' (Morant and Jørgensen, 2008). In intact plant cells, CNGs and their catabolic enzymes are stored in separate compartments, but are brought into contact upon tissue disruption, caused e.g. by chewing or physical processes such as maceration or freezing during food processing (Gleadow and Woodrow, 2002). The strategy of handling CNGs and their catabolic enzymes as a binary system endows plants with an effective defence against generalist herbivores. CNGs are therefore referred to as 'phytoanticipins'. Additionally, CNGs are believed to represent a pool of nitrogen to be used by the plant if needed (Gleadow and Møller, 2014).

The hydrolysis of CNGs can involve various enzymes. In addition to the plant enzymes mentioned above, β-glucosidases located in the mammalian intestinal epithelium and in colonic bacteria appear to play an important role (see Section 3.1.1.1). The cyanide eventually released in the mammalian gastrointestinal tract is detoxified through various pathways (see Section 3.1.1.1), and the toxicity of amygdalin or any other CNG primarily depends on the rate of cyanide liberation versus cyanide detoxification.

Hydrocyanic acid is also named hydrogen cyanide, formonitrile, methanenitrile or prussic acid, among others. It has the chemical formula HCN, the molecular mass 27.03 g mol^{-1} and the CAS number 74-90-8. In pure form, it is a colourless liquid with a boiling point of 25.6°C and a melting point of −14°C. Its density is 0.687 g mL^{-1} and its vapour pressure is 630 mmHg at 20°C. It is completely miscible with water or ethanol. HCN is a very weak acid with a pK_a of 9.2 and a pK_b of 4.8, and aqueous solutions of its alkali salts (cyanides) are therefore quite alkaline. HCN vapours have a characteristic odour resembling bitter almond oil, but a high percentage of the population does not readily smell HCN due to their genetic disposition.

1.3.2. Analytical methods

This article does not provide a full list of potential methods to quantify the concentration of amygdalin or cyanide (originating from amygdalin) in apricot kernels and products derived from apricot kernels. Rather, the intention is to identify methods that are used as the standard methods of analysis.

1.3.2.1. Quantification of amygdalin

The extraction step from food samples is one crucial aspect of any analytical procedure due to the potential of amygdalin for rapid degradation. It has been concluded that the extraction efficiency for CNGs could vary considerably depending on the grinder and the extraction methods. In addition to enzymatic degradation, amygdalin can epimerise to neoamygdalin in hot aqueous solutions (e.g. Wasserkrug and Rassi, 1997; Hwang et al., 2002; Koo et al., 2005). Lee et al. (2013) reported that approximately 35% of a 200 μg/kg amygdalin standard in methanol/water (40:60 v/v) converted to neoamygdalin during the 24 h extraction process with methanol at room temperature. This might lead, in some cases, to substantial underestimation of the amount of CNG. Water, water containing 0.1% citric acid, methanol or ethanol are frequently used for amygdalin extraction (e.g. Lv et al., 2005; Bolarinwa et al., 2014). High-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (HPLC-UV) and HPLC with diode-array detection (HPLC-DAD) have been widely applied to quantify amygdalin in food samples after extraction. More recently, solid-phase extraction along with liquid chromatography (LC)-tandem mass spectrometry (LC-MS/MS) analysis has been used, improving both sensitivity and selectivity of the analyses (e.g. Lee et al., 2013). To selectively separate amygdalin and neoamygdalin, Wahab et al. (2015) recommended the use of cyclodextrin chiral columns or porous graphitic carbon columns. These authors demonstrated that the conversion of amygdalin to neoamygdalin is glassware dependent and thus they recommended inert plastic containers for storage of aqueous amygdalin solutions. Notably, in their study, commercial preparations of amygdalin contained greater quantities of neoamygdalin and ∼ 5% of other degradation products. Besides LC-based techniques, less frequently gas chromatography mass spectrometry (GC-MS) as well as enzyme-linked immunosorbent assays (ELISAs) have been applied to quantify amygdalin in food (FAO/WHO, 2012; Bolarinwa et al., 2014).

1.3.2.2. Quantification of cyanide (originating from amygdalin)

Crucial steps in the analysis of cyanide (originating from amygdalin) include sample handling and complete hydrolysis of CNGs. Hydrolysis can be achieved by acid catalysis or enzymatic degradation. For the latter, commercial glycosidases or enzyme preparations from animals and microorganisms are frequently used (EFSA, 2007a). To ensure that all released HCN is retained for analysis, food samples

should be incubated with the enzymes or the diluted acid in sealed containers. Methods of quantifying the released cyanide include colorimetry, spectrophotometry and chromatography with subsequent detection (FAO/WHO, 2012).

In the enzyme-based picrate method, HCN is liberated via exogenous enzymes and allowed to react directly with sodium picrate paper strips suspended above the solution. After removing and immersing the paper in water, the absorbance is measured (510 nm) to calculate the cyanide content. Haque and Bradbury (2002) compared cyanide determination in 10 plants and food samples comparing the enzyme-based picrate method and an acid hydrolysis method. The acid hydrolysis method detected cyanide via a formed polymethine dye (600 nM) after the addition of chloramine-T and barbituric acid. They concluded that the applied acid hydrolysis method is more difficult to use as well as less accurate and reproducible than the picrate method.

The official method of the Association of German Agricultural Analytic and Research Institutes for cyanide determination in food and feed is based on acid hydrolysis, subsequent distillation of liberated cyanide and titration (iodide/silver nitrate) (VDL-UFA, 1976).

Recently, FSANZ (2014) compared cyanide analysis in food samples via acid hydrolysis followed by colorimetric detection of cyanide with a standardised HPLC with a fluorescence detection-based method for animal feeding materials (EU, 2012). The approved method involves extraction of CNGs with an acid solution, enzymatic digestion applying β-glucosidase, collection of cyanide in a potassium hydroxide solution by steam distillation, derivatisation of cyanide with taurine and 2,3 naphthylene dicarboxy aldehyde and subsequent analysis of the fluorescent cyanide complex by HPLC with fluorescence detection.

The authors concluded that the acid hydrolysis method is not appropriate for the measurement of very low cyanide concentrations [limit of detection (LOD) 1.5–5 mg HCN/kg]. However, they considered the acid hydrolysis robust for samples with high cyanide concentrations such as apricot kernels. For the sensitive EU method (LOD 0.1 mg HCN/kg) to be applied for food samples with high concentrations such as bitter apricot kernels, extracts need to be diluted substantially to bring them in the measuring range (FSANZ, 2014).

1.3.3. Previous assessments

A total of three EFSA opinions pertinent to the current risk assessment have been published previously. In the opinion on HCN in flavourings and other food ingredients with flavouring properties, it was described that quantitative data on oral acute toxicity of HCN are limited to studies in dogs and rats in which the medium lethal dose (LD_{50}) was found to be 5.3 mg potassium cyanide (KCN)/kg body weight (bw) and 10–15 mg KCN/kg bw equivalent to 2.13 and 4.0–6.03 mg CN/kg bw, respectively. The lowest published lethal dose to humans is 0.56 mg HCN/kg bw. No safe intake level with respect to the acute toxicity of HCN was established. The peak plasma levels of HCN determine the acute toxicity. In case of intake of CNGs, HCN is slowly and incompletely released by hydrolysis by gut micro flora and then absorbed, and the acute toxicity for HCN will be lower than if injected. The lethal oral dose of the CNG linamarin in rat was determined to be 450 mg linamarin/kg bw. It was concluded that the estimated exposure to cyanide from flavouring ingredients (at the 97.5th percentile of 3.6 μg/kg bw per day) was unlikely to give rise to acute toxicity in humans (EFSA, 2004).

As HCN is one of several precursors of ethyl carbamate formation, EFSA also considered this compound in the opinion on the presence of ethyl carbamate and HCN in food and beverages (EFSA, 2007a). However, for risk assessment purposes and conclusions, direct reference was made to the opinion on HCN in flavourings and other food ingredients with flavouring properties (EFSA, 2004).

In another EFSA opinion on cyanogenic compounds as undesirable contaminants in animal feed, specific values in the lower mg HCN equivalents per kg body weight per day range were defined that were considered tolerable in a number of species. This report also mentioned intravenous (i.v.) LD_{50} values for several species, ranging from 0.78 mg CN/kg bw for rat to 1.38 mg CN/kg bw for guinea pig (EFSA, 2007b).

In their assessment, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the data from human epidemiological studies were insufficient to form the basis for establishing a health-based guidance value. Accordingly, JECFA used an animal developmental toxicity study as the basis for deriving an acute reference dose (ARfD) for the CNG linamarin. Benchmark dose modelling of the data from that particular study provided a 95% lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) for linamarin of 85 mg/kg bw for increased skeletal defects in developing hamster

foetuses following acute exposure of maternal animals. While this study did not use dietary exposure, gavage dosing was considered relevant to establishing the ARfD. Following application of a 100-fold uncertainty factor (UF), JECFA established an ARfD for linamarin of 0.9 mg/kg bw equivalent to 0.09 mg/kg bw as CN. When compared on a CN molar basis, this value was considered to also be applicable to other CNGs. Therefore, JECFA recommended conversion of the ARfD for linamarin to a CN equivalent dose of 0.09 mg/kg bw. This CN equivalent ARfD applies only to foods containing CNGs as the main source of CN. JECFA (FAO/WHO, 2012) also established a Provisional Maximum Tolerable Daily Intake (PMTDI) of 20 μg/kg bw CN based on findings in a 13-week toxicity study on sodium cyanide conducted by the US National Toxicology Program (NTP).

The UK Committee on the Toxicity of Chemicals in Food, Consumer Products and Environment (COT) proposed an ARfD of 5 μg CN/kg bw, which was obtained by dividing the acute lethal dose of CN in humans (i.e. 0.5–3.5 mg/kg bw) by 100. This UF comprised a factor of 10 for interindividual variability and another factor of 10 to extrapolate from an effect level to a no-effect level because of the steep dose–response relationship. Bitter apricot kernels typically contain approximately 0.5 mg CN/kernel. Thus, consumption of 1 kernel/day would result in a CN intake of 0.5 mg/day, which is equivalent to 8 μg CN/kg bw for an adult weighing 60 kg and that approximates the proposed ARfD (COT, 2006).

The Hellenic Food Authority (EFET) also considered 0.5 mg CN per bitter apricot kernel and a PMTDI of 12–20 μg CN/kg bw, which is based on the TDI set by the WHO (1993) in the context of developing drinking water guidelines (WHO, 2003) and the JECFA PMTDI (FAO/WHO, 2012). On the basis of this information, EFET recommended that the maximum daily intake for adults is up to two bitter apricot kernels and that children as well as pregnant women and breastfeeding mothers should avoid consuming bitter apricot kernels. Given the fluctuations in the concentration of CNGs in bitter apricot kernels, EFET advises food companies to mention on the product packaging the maximum quantity that can be safely consumed (EFET, 2014).

To determine the risk associated with the estimated dietary exposures, the Food Standards Australia New Zealand (FSANZ) used an ARfD of 80 μg HCN/kg bw, which was obtained from their previous assessment on HCN in cassava chips. The principal CNG in cassava is linamarin. For linamarin, an ARfD of 700 μg/kg bw was established on the basis of death in hamsters at doses greater than 70 mg/kg bw and applying a 100-fold UF to account for intraspecies variability and interspecies extrapolation. The available data indicate that no unchanged linamarin is excreted in the *faeces* following oral ingestion, thus suggesting that there is sufficient enzymatic capacity in the microflora of the *caecum* to completely hydrolyse large amounts of linamarin. As total HCN levels are more readily determined than linamarin levels, the linamarin ARfD was converted to an ARfD for total HCN measured in cassava as an analytical convenience. The linamarin ARfD equates to an ARfD for HCN in cassava of 80 μg HCN/kg bw (FSANZ, 2008). For use with respect to apricot kernels, a distinction was made between kernels with or without skin, containing 2,820 and 440 mg HCN/kg kernel, respectively. Assuming that one kernel weighs approximately 0.6 g, specific recommendations were made with respect to maximum daily consumption per age class (FSANZ, 2014).

The German Federal Institute for Risk Assessment (BfR) assumed that bitter apricot kernels contain up to 4 mg chemically bound cyanide per kernel, which corresponds with 1.5 mg of cyanide per medium-sized apricot kernel and up to 3.0 mg cyanide per large apricot kernel. Keeping in mind that the fatal dose of cyanide in humans is around 0.5–3.5 mg/kg bw, BfR derived an ARfD of 75 μg cyanide/kg bw. This dose (i.e. 4.5 mg for a bw of 60 kg) is safe when a meal is eaten and is equivalent to approximately two large bitter apricot kernels in adults. The ARfD was derived from a study in human volunteers after consumption of foods containing high levels of CNGs, including bitter apricot kernels, containing equivalents of 6.8 mg CN. The highest peak level of cyanide in blood was 31.9 μM in a person weighing 60 kg after consumption of cassava. In this individual, 6.8 mg CN corresponded to a dose of 0.113 mg/kg bw. Roughly, two thirds of this dose (i.e. 75 μg CN/kg bw) would have resulted in a CN peak level of 20 μM (which was assumed to be the threshold for toxicity) in this person. The application of any further safety factors was not judged to be necessary, as peak levels were found to be mainly determined by the body weight defining the volume of distribution, whereas mechanisms of elimination, such as activity of rhodanese with possible higher variability, are unimportant for peak levels in case of a bolus application of a relatively high dose of CN (BfR, 2015; Abraham et al., 2016).

1.3.4. Legislation

Council Regulation (EEC) No 315/93[5] stipulates that food containing a contaminant in an amount unacceptable for public health shall not be placed on the market, that contaminant levels should be kept as low as can reasonably be achieved and that, if necessary, the EC may establish maximum levels for specific contaminants. These maximum levels are laid down in the Annex of Commission Regulation (EC) No 1881/2006[6] and may include limits for the same contaminants in different foods, analytical detection limits and reference to the sampling and analysis methods to be used. There are no EU maximum levels established for cyanide in raw apricot kernels and/or products thereof.

Regulation (EC) No 1334/2008[7] governs the use of flavourings and food ingredients with flavouring properties in foods. The regulation also provides maximum levels of certain substances naturally present in flavourings and food ingredients with flavouring properties. A maximum level for HCN of 50 mg/kg has been established for nougat, marzipan or its substitutes or similar products, of 5 mg/kg in canned stone fruits and of 35 mg/kg in alcoholic beverages.

Regulation (EC) No 110/2008[8] governs the definition, description, presentation, labelling and protection of geographical indications of spirit drinks and establishes a maximum content of HCN of 7 g/hL of 100% volume alcohol (70 mg/L) in stone-fruit marc spirits and stone-fruit spirits.

Cyanogenic glycosides are mentioned in medicines legislation of some Member States. For example, the UK Human Medicines Regulations 2012[9] stipulate that cyanogenic substances, other than preparations for external use, shall only be available on prescription. Cyanogenic substances are defined therein as preparations which are presented for sale or supply under the name of, or as containing, amygdalin, laetrile, vitamin B17, or contain more than 0.1% by weight of any substance having the formula either of α-cyanobenzyl-6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside, or α-cyanobenzyl-β-D-glucopyranosiduronic acid. Medicinal products containing amygdalin or its derivatives mandelonitrile, mandelonitrile-glycoside or laetrile are not authorised in Germany. According to the Federal Institute for Drugs and Medical Devices, such medical products are to be classified as dangerous according to paragraph 5.2 of the Medicinal Products Act[10] and must not be placed on the market or be applied to individuals even if a medical prescription is available.

In Australia and New Zealand, raw apricot kernels which are defined as hulled, dehulled, blanched, ground, milled, cracked, chopped or whole kernels of *Prunus armeniaca* must not be sold in a retail sale and must not be used as an ingredient in food unless they have been processed rendering them safe for human consumption.[11]

2. Data and methodologies

2.1. Data

2.1.1. Occurrence data

EFSA gathers at the EU-level data on the occurrence of chemical substances in food and feed through continuous collections and/or calls for particular assessments. No data concerning cyanide occurrence in apricot kernels were available in the EFSA occurrence database. A literature review was carried out in order to collect published data. Collection and appraisal of occurrence data was carried out as described in Section 2.2.2.

[5] Council Regulation (EEC) No 315/93 of February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1–5.
[6] Regulation (EC) No 1881/2006 of the European Parliament and the Council of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.
[7] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 160/1, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC (OJ L 354, 31.12.2008, p. 34–50).
[8] Regulation (EC) No 110/2008 of the European Parliament and of the Council of 15 January 2008 on the definition, description, presentation, labelling and the protection of geographical indications of spirit drinks and repealing Council Regulation (EEC) No 1576/89. OJ L 39, 13.2.2008, p. 16.
[9] http://www.legislation.gov.uk/uksi/2012/1916/pdfs/uksi_20121916_en.pdf
[10] http://www.gesetze-im-internet.de/amg_1976/BJNR024480976.html
[11] http://www.foodstandards.gov.au/code/changes/gazette/Documents/GazetteNo159WebVersion.pdf

2.1.2. Consumption data

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country. This is described in the guidance on the use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011). New consumption surveys have been recently added in the Comprehensive Database (at the end of 2014).

Within the Comprehensive Database, only one subject, a child (body weight of 15 kg) in a survey conducted in Bulgaria, reported the consumption of 50 g (3.3 g/kg bw) of apricot kernels, without specifying if bitter or sweet, nor if treated prior to consumption. Therefore, data available in the Comprehensive Database concerning the consumption of apricot kernels were insufficient to estimate the dietary exposure to cyanide in the current assessment.

Consequently, websites promoting the consumption of apricot kernels and products thereof were searched for an overview of the promoted amounts of apricot kernels to be ingested.

2.1.3. Toxicokinetic and toxicological data

All data were obtained as described in Section 2.2.1.

2.2. Methodologies

2.2.1. Collection and appraisal of occurrence, toxicokinetics and toxicity data

In 2004, EFSA published a scientific opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) on HCN in flavourings and other food ingredients with flavouring properties (EFSA, 2004). It was assumed that all relevant literature until that date was considered for this previous assessment. Literature (no time limit was applied) referenced therein on chemistry and analysis, metabolism and kinetics, food processing and oral acute-, and sub-acute toxicity of cyanide, amygdalin and apricot kernels has been evaluated, and considered or dismissed by expert judgement for the present assessment (a total of 53 publications). Studies on genotoxicity and chronic toxicity, on CNGs not present in apricot kernels (such as linamarin present in cassava) and on cassava were not considered. Any limitations of the information used are documented in this opinion.

In 2007, EFSA published a scientific opinion on cyanogenic compounds as undesirable substances in animal feed (EFSA, 2007b). Likewise have publications referenced therein been considered for the present assessment following selection criteria as described above, yielding a total of additional 16 publications to be considered. In the same year EFSA published a scientific opinion on ethyl carbamate and HCN in food and beverages (EFSA, 2007a). For the hazard characterisation in this opinion, EFSA referred to the conclusions of the previous EFSA opinion of the AFC Panel (EFSA, 2004) and additional data that had become available since then. Upon reviewing the references in this opinion applying the selection criteria described above, no further publications relevant for the present assessment have been identified.

In order to retrieve relevant information made available after publication of the first EFSA opinion dealing with cyanide (EFSA, 2004), the CONTAM Panel retrieved and appraised literature made publicly available between January 2004 and June 2015. A systematic and comprehensive search for literature was conducted for peer-reviewed original research pertaining to HCN in raw apricot kernels and products derived thereof. The search strategy was designed to identify scientific literature dealing with analytical determination, chemistry, formation, occurrence and exposure, metabolism and toxicity of HCN. In addition, literature covering food and processing was included. The search terms HCN and cyanide were excluded from the search, due to a high number of scientific literature covering non-cyanide-related uses of the abbreviation HCN and non-apricot kernel-related cyanide. All search terms and Boolean operators used are presented in detail in Appendix A. In addition to HCN, the search string covered any other potential term used, such as CNGs, amygdalin, prunasin, prussic acid, laetrile, formonitrile and vitamin B17. Literature search was not restricted to publications in English language; however, literature in other languages was only considered if an English abstract was

available. The literature search was performed on 4 June 2015. Web of Science[12] and Pubmed[13] were identified as databases appropriate and exhaustive for retrieving literature for the present evaluation.

The references resulting from the literature search were imported and saved using a software package (EndNote[14]), which allows effective management of references and citations. Of the 4,117 retrieved citations, duplicate references were removed using the EndNote software. The remaining 1,733 references were screened using title and abstract to identify the relevant literature. Using expert judgement, 75 publications were identified as relevant to apricot kernels and considered for the present assessment.

In addition to this first search, a second search was carried out to include the search terms 'bitter apricot' and 'apricot paste' covering the period 1 January 2004–4 September 2015 and covering all topics and using the same Boolean operators as in the initial search. Of the 731 references retrieved, duplicates were removed using the EndNote software, and the remaining 401 references were screened using title and abstract (see Appendix A). Upon appraisal of the studies following inclusion/exclusion criteria as described above and by applying expert judgement, an additional 17 publications were identified as relevant, retrieved and considered for the assessment.

In addition, other previous scientific evaluations by national or international bodies were also screened for relevant information.

These were:

- Hydrogen cyanide, sodium cyanide and potassium cyanide (DECOS, 2002)
- Hydrogen cyanide and cyanides: Human health aspects (WHO, 2004)
- Statement on cyanogenic glycosides in bitter apricot kernels (COT, 2006)
- Toxicological profile for cyanide (ATSDR, 2006)
- Toxicological review of HCN and cyanide salts (US EPA, 2010)
- Safety evaluation of certain food additives and contaminants: Cyanogenic glucosides (FAO/WHO, 2012)
- Survey of cyanogenic glycosides in plant-based foods in Australia and New Zealand 2010–2013 (FSANZ, 2014)
- Risk assessment of the presence of CN ions in bitter almonds and apricot kernels for consumer health and recommendations for consumption of such products by adults and children (EFET, 2014)
- Two bitter apricot kernels per day are the limit for adults – children should avoid them altogether – updated opinion No 009/2015 of 7 April 2015 (BfR, 2015; Abraham et al., 2016).

These assessments and the publications referenced therein were considered when relevant using the inclusion/exclusion criteria described above and applying expert judgement.

During the development of the opinion, additional relevant publications not retrieved in the above-mentioned literature (e.g. studies relevant to the assessment on receptor binding) assessment have been identified and considered for the assessment.

2.2.2. Methodology applied for hazard assessment

The CONTAM Panel applied the general principles of the risk assessment process for chemicals in food as described by WHO (2009), which include hazard identification and characterisation, exposure assessment and risk characterisation. Additionally to the principles described by WHO (2009), EFSA guidance pertaining to risk assessment has been applied for the present assessment. In brief, the EFSA guidance covers the procedures currently used within EFSA for the assessment of dietary exposure to different chemical substances and the uncertainties arising from such assessments. For details on the specific EFSA guidances applied, see Appendix B.

[12] Web of Science (WoS), formally ISI Web of Knowledge, Thomson Reuters (http://thomsonreuters.com/thomson-reuters-web-of-science/).

[13] PubMed, Entrez Global Query Cross-Database Search System, National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM), Department of the National Institutes of Health (NIH), United States Department of Health and Human Services (http://www.ncbi.nlm.nih.gov/pubmed/).

[14] EndNote X5, Thomson Reuters. http://endnote.com/

2.2.3. Exposure assessment

Following EFSA guidelines (EFSA Scientific Committee, 2012), the Panel decided to estimate the exposure to cyanide via the consumption of raw apricot kernels separately for toddlers and adults. This encompasses dietary exposures of older children and adolescents, which are generally likely to be intermediate between those of adults and toddlers (EFSA Scientific Committee, 2012). For 'Adults' (18–64 years old), a standard weight of 70 kg was used, while for the group 'Toddlers' (aged 1–3 years), a weight of 12 kg was considered (EFSA Scientific Committee, 2012).

Due to the lack of consumption data, the Panel decided to perform an exposure assessment using the highest portion size promoted from the websites promoting the consumption of apricot kernels for the general population and highest portion size promoted for cancer patients (see Section 3.2).

Potential exposure to cyanide (originating from amygdalin) due to the consumption of apricot kernels in the general population and in cancer patients for toddlers and adults was estimated through the following scenarios:

1) In the first scenario, it was assumed that the apricot kernels' cyanide concentration was equal to the lowest cyanide mean concentration level available in the literature for bitter apricot kernels.
2) In the second scenario, it was assumed that the apricot kernels' cyanide concentration was equal to the upper cyanide mean concentration level available in the literature for bitter apricot kernels (see Section 3.3).

Mean values were considered appropriate in both scenarios as the cyanide content of individual kernels varies and if a large number of kernels are consumed, it is unlikely that all will contain cyanide at the maximum reported level (see also Section 3.3).

In both exposure scenarios, the midpoint of the mean weight for apricot kernels reported in the literature was used (see Section 3.3).

3. Assessment

3.1. Hazard assessment

3.1.1. Toxicokinetics and metabolism

The health hazard posed by apricot kernels is due to cyanide. As described earlier (Section 1.3.1), intact apricot kernels do not contain free cyanide but the CNG amygdalin. Upon mechanical or thermal disruption of the kernel tissue, plant enzymes are set free which hydrolyse amygdalin to prunasin and eventually lead to the release of HCN (Figure 3). Complete hydrolysis of 1 g of amygdalin generates 59.0 mg of HCN (FAO/WHO, 2012).

Orally ingested apricot kernels which have been chewed or processed may contain a mixture of compounds ranging from amygdalin to free HCN and cyanide ions, including the intermediates of degradation. Because the degrading plant enzymes, i.e. amygdalin hydrolase (AH), prunasin hydrolase (PH) and mandelonitrile lyase (MNL) (Figure 3), are presumably inactivated by the acidic pH of the stomach, the components of the mixture can either be absorbed as such or after metabolism by mammalian or bacterial enzymes present in the gastrointestinal tract.

The toxicokinetics and metabolism of cyanides have been well studied, because these agents are important industrial chemicals as well as military and environmental toxins, occurring e.g. in cigarette smoke and as combustion products of nitrogen-containing plastics. In comparison, little is known about the toxicokinetics of amygdalin and prunasin and their intermediate degradation products.

3.1.1.1. Laboratory animals

Toxicokinetic studies in laboratory animals have been performed with pure amygdalin, prunasin or cyanide. One study with mandelonitrile in horses has been identified.

When a single dose of 50 mg amygdalin/kg bw was administered by oral gavage to young Beagle dogs of both sexes, plasma levels and urinary excretion of unchanged amygdalin were very low, and the systemic availability was estimated to be only 2.2 ± 0.3% of the dose (Rauws et al., 1982). In contrast, an appreciable quantity (21 ± 9% of the dose) was excreted in the 6-h urine as prunasin. Likewise, after oral administration of the same dosage of amygdalin to male and female Wistar rats, 0.8 ± 0.5% of the dose was excreted unchanged and 39 ± 9% as prunasin with the urine (Rauws

et al., 1982). When the same dose of amygdalin was given to dogs by the i.v. route, 71 ± 8% of the dose was excreted unchanged and < 0.2% as prunasin in the 6-h urine. This study implies that the absorption of intact amygdalin in the gastrointestinal tract is rather limited, but part of the amygdalin appears to be hydrolysed to prunasin in the intestine. In support of this notion, an *in vitro* study using isolated perfused segments of rat small intestine showed an appreciable hydrolysis of amygdalin to prunasin in the proximal jejunum (Strugala et al., 1986). There were no indications of the further metabolism of prunasin to benzaldehyde. The hydrolysis of amygdalin to prunasin was also observed in incubations with mucosal homogenates from the upper small intestine. It was pH-dependent and could be inhibited by glucono-δ-lactone, which is known as a potent inhibitor of lysosomal β-glucosidase activity in the rat intestine. The common glycosylases of the mammalian intestine, e.g. α-amylase and α-glucosidase, and mammalian liver, e.g. β-glucuronidase and β-galactosidase, did not catalyse the hydrolysis of amygdalin to prunasin. Moreover, no hydrolysis of amygdalin or prunasin could be observed in isolated perfused rat liver. However, the faecal contents of rats hydrolysed both amygdalin and prunasin, and the released mandelonitrile disintegrated spontaneously into cyanide and benzaldehyde (Strugala et al., 1986). The authors concluded that amygdalin itself is not absorbed to an appreciable amount in the gastrointestinal tract, but hydrolysed to prunasin in the jejunum, which is then absorbed and subsequently excreted in the urine without releasing appreciable amounts of cyanide. The toxicity of amygdalin and prunasin due to the release of cyanide appeared to require the enzymatic activity of the gut flora.

A later *in vitro* study (Strugala et al., 1995) also indicated that prunasin is transferred intact, i.e. without hydrolysis and formation of cyanide, across the intestinal wall of the rat jejunum and ileum. The transfer in the jejunum exhibited saturation and interfered with the uptake of methyl α-D-glucoside, implying the participation of a glucose transporter. Transfer of prunasin in the ileum exhibited characteristics of passive diffusion. When Wagner and Galey (2003) studied the effect of amygdalin and prunasin on the transport of glucose in the rat jejunum, prunasin, but not amygdalin, was found to reduce the influx of glucose, consistent with the hypothesis that only prunasin is carried across the gut mucosa via the epithelial sodium-dependent monosaccharide transporter SGLT1.

Consistent with the virtual lack of absorption of intact amygdalin and the metabolism to prunasin is the finding by Chen et al. (2012) that no amygdalin but a delayed peak of prunasin could be found by HPLC-UV analysis in rat plasma after oral gavage dosing of amygdalin.

Convincing evidence for an important role of the gastrointestinal micro flora in the release of cyanide from amygdalin was provided by Carter et al. (1980). Adult conventional Sprague-Dawley rats, i.e. rats with an intact bacterial flora, and germfree rats, which lack this flora, received the same dose of amygdalin (600 mg/kg bw) by gastric intubation. The germfree rats did not have detectable levels of cyanide in the blood and did not exhibit symptoms of cyanide intoxication, while the conventional rats had high levels of cyanide and some died within a few hours. Levels of thiocyanate, the major metabolite of cyanide (see below), paralleled that of cyanide. When amygdalin was incubated with the caecal contents of conventional rats, ca. 40% of the amygdalin was degraded after 24 h with the concomitant formation of benzaldehyde, while no degradation was observed with the caecal content of germfree rats.

The importance of the gut microbiota for the release of cyanide from orally dosed amygdalin was also demonstrated by Newton et al. (1981). When female Fischer 344 rats were pretreated with the antibiotic neomycin prior to an oral dose of amygdalin, almost no thiocyanate was detectable in the 24-h urine, while rats with an intact bacterial flora excreted a large amount of thiocyanate under the same conditions. In the same study, various segments of the rat gastrointestinal tract were incubated with amygdalin and the generation of cyanide was measured. The highest activity for cyanide formation was observed for the rat caecum and the lowest for the rat stomach.

Frakes et al. (1986) studied cyanide liberation during incubation of equimolar concentrations of amygdalin and prunasin with a crude preparation of β-glucosidase obtained from the pooled caeca and their contents of female golden hamsters. Prunasin was hydrolysed more than twofold faster than amygdalin. When amygdalin was administered to female hamsters at a dose of 0.44 mmol/kg bw (corresponding to 200 mg/kg bw) by oral gavage, the blood cyanide concentration reached a transient plateau (at about 150 nmol/mL) and was accompanied by symptoms of cyanide poisoning in most animals (Frakes et al., 1986).

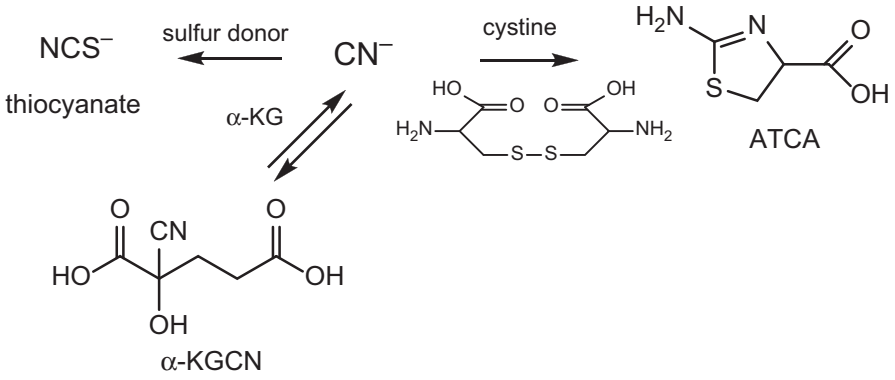
Majak and Cheng (1987) reported that rumen fluid from cattle also degraded amygdalin and prunasin to cyanide. The rate of cyanogenesis was found to depend on the cattle diet but was always about twofold higher for prunasin than for amygdalin. Thirty out of 68 common strains of anaerobic rumen bacteria were shown to exhibit cyanogenic activity (Majak and Cheng, 1984).

When mandelonitrile was orally administered at a dose of 3 mg/kg bw to horses, peak blood concentrations of cyanide (about 70 mM) were observed as early as 3 min after dosing, followed by a rapid decline; no signs of cyanide intoxication or distress were observed, and the oral bioavailability of cyanide from mandelonitrile was 57% (Dirikolu et al., 2003).

The animal studies outlined above shown that orally administered amygdalin is virtually not absorbed *per se*, but partly hydrolysed by mammalian β-glucuronidase in the jejunum to prunasin, which is then absorbed utilising the glucose transporter. Amygdalin and prunasin reaching the caecum are efficiently hydrolysed by the bacterial microflora, resulting in the release of cyanide. For the toxicity of CNGs, the liberation of cyanide in the caecum appears to be of critical importance.

As HCN is a very weak acid with a pK_a of 9.2, cyanide-containing material leads to non-dissociated HCN in every part of the gastrointestinal tract. Non-dissociated HCN is a small and non-polar molecule which is readily absorbed through the gastric and intestinal mucosa. In the blood, most of the cyanide is confined to the erythrocytes where it binds to methaemoglobin (Vesey et al., 1979; Lundquist et al., 1985). The proportion of HCN escaping first-pass metabolism in the liver (see below) is rapidly distributed via the systemic circulation into all tissues. A complete analysis of the body distribution in cadavers of animals and humans poisoned with cyanide showed roughly 50% of the absorbed cyanide to be in the blood, about 25% in muscles and 25% in all the other organs together, predominantly in the liver and brain (Schulz, 1984). After oral administration of a single dose of 3.0 mg KCN/kg bw to rats, pigs and goats, the half-life of cyanide in blood was 0.64, 0.54 and 1.28 h and the volume of distribution was 0.35, 0.29 and 0.41 L/kg, respectively (Sousa et al., 2003). In another study with rats, the peak blood level of cyanide was observed 2 min after intragastric administration of 1 mg KCN/kg bw, and a blood elimination half-life of 0.23 h was calculated (Leuschner et al., 1991).

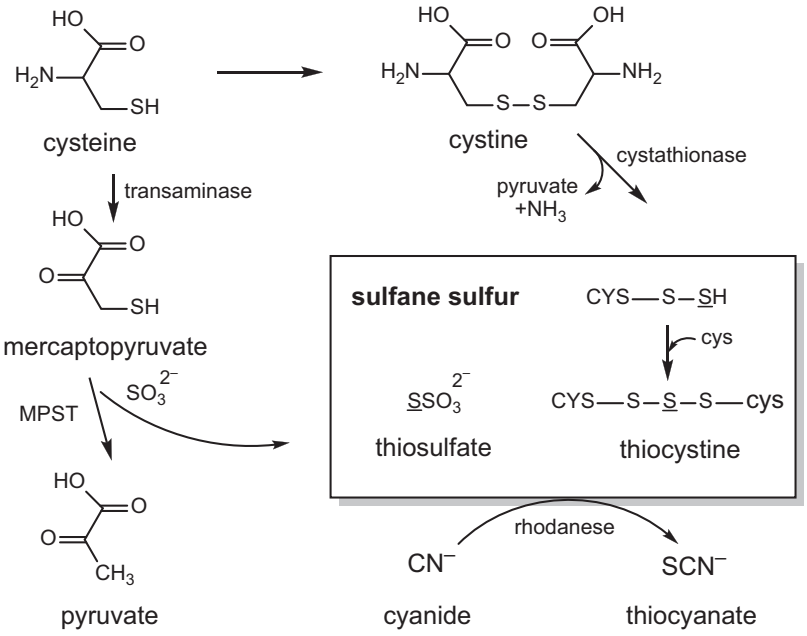
The mammalian organism has developed several metabolic pathways in order to detoxify cyanide (Figure 4). In the presence of a sulfur donor, e.g. thiosulfate, and a sulfur transferase, e.g. rhodanese (see below), about 70% of a dose of cyanide is metabolised to thiocyanate (Sousa et al., 2003). The transformation to thiocyanate represents a detoxification of cyanide, because thiocyanate does not block the electron transport in the mitochondrial respiratory chain. The acute toxicity of thiocyanate is about 100-fold lower than that of cyanide, based on the oral LD_{50} in rats (Bilska-Wilkosz et al., 2015). Cyanide can also react with L-cysteine through the putative intermediate β-thiocyanoalanine to 2-amino-2-thiazoline-4-carboxylic acid (ATCA). This pathway accounts for about 15–20% of cyanide metabolism (Wood and Cooley, 1956). Thiocyanate and ATCA are chemically stable metabolites which are not further metabolised but excreted with the urine (Bhandari et al., 2014). Another detoxification pathway is the reaction of cyanide with endogenous α-ketoglutarate (α-KG) to form α-ketoglutarate cyanhydrin (α-KGCN). This pathway is assumed to become important when the thiocyanate and ATCA pathways are overwhelmed (Mitchell et al., 2013). Other minor pathways, which are of interest primarily as biomarkers for exposure (see Section 3.1.1.3), have also been described, e.g. the reaction with cysteine disulfide groups in serum albumin (Fasco et al., 2007, 2011). In addition to binding to methaemoglobin, cyanide binds to hydroxocobalamin (vitamin B_{12b}). The complex of cyanide with hydroxocobalamin is excreted with the urine. This binding can be used in the treatment of cyanide poisoning by inducing methaemoglobinaemia or administering hydroxocobalamin (Way, 1984; Petrikovics et al., 2015).



ATCA: 2-amino-2-thiazoline-4-carboxylic acid; α-KG: α-ketoglutarate; α-KGCN: α-ketoglutarate cyanhydrin.

Figure 4: Detoxification of cyanide ions

Whereas the formation of ATCA and α-KGCN proceeds without the help of enzymes, the primary detoxification pathway of cyanide, i.e. formation of thiocyanate, appears to be mediated by three enzymes (see Figure 5).



MPST: mercaptopyruvate sulfurtransferase. See text in this section for further explanation.

Figure 5: Various pathways leading to compounds with sulfane sulfur

The first enzyme is thiosulfate:cyanide sulfurtransferase (EC 2.8.1.1), also termed rhodanese, which transfers sulfur from a sulfane sulfur source (see below) such as thiosulfate to cyanide (Wróbel et. al., 2004). The second enzyme, i.e. 3-mercaptopyruvate:cyanide sulfurtransferase (EC 2.8.1.2, MPST), catalyses the transfer of sulfur from 3-mercaptopyruvate to a variety of sulfur acceptors, including sulfite and cyanide (Jarabak and Westley, 1980). Thereby, MPST can provide thiosulfate to rhodanese (Fig. 5), but also directly convert cyanide to thiocyanate. 3-Mercaptopyruvate is formed through transamination of cysteine. The third enzyme, i.e. cystathione γ-lyase (EC 4.4.1.1., cystathionase), converts cysteine to thiocysteine and thiocysteine, which also serve as sulfane sulfur donor substrates for rhodanese (Baskin et al., 1999). Sulfane sulfur is a divalent sulfur atom covalently bound to another sulfur atom, and most sulfane sulphur-containing compounds are endogenous metabolites formed from cysteine. Bilska-Wilkosz et al. (2015) recently reported that lipoic acid, a naturally occurring dithiol compound, increased the rate of thiocyanate excretion in rats dosed with cyanide, suggesting that lipoic acid may also be involved in cyanide detoxification.

Rhodanese is a ubiquitous enzyme present in many tissues of humans and other species (Drawbaugh and Marrs, 1987). The highest activities are commonly measured in the liver and kidneys. However, in sheep and cattle, the epithelium of rumen, omasum and reticulum has the highest activity (Aminlari and Gilanpour, 1991). Within the cell, rhodanese is located predominantly in the mitochondria. In humans, polymorphic variants of rhodanese have been characterised (Libiad et al., 2015). Species differences in rhodanese activity have been reported (e.g. Drawbaugh and Marrs, 1987; Aminlari and Gilanpour, 1991), but cannot be directly correlated with the sensitivity to cyanide because of the participation of other enzymes and pathways in cyanide detoxification. Moreover, the availability of sulfur donors is of paramount importance for the rate of detoxification of cyanide. The major sulfur donors are the sulfur-containing amino acids cysteine and methionine, which provide the sulfur to form thiosulfate from sulfite in the cells. Orally administered thiosulfate is very poorly absorbed from the gastrointestinal tract, and even after i.v. administration remains mostly in the extracellular space (Schulz, 1984). In support of the importance of dietary sulfur amino acids for cyanide detoxification, Kimani et al. (2014) observed a markedly lower rate of cyanide detoxification in the spinal cord of rats after feeding a diet lacking 75% of sulfur amino acids as compared to a normal diet.

3.1.1.2. Humans

Because of the putative anticancer activity of amygdalin, most toxicokinetic studies in humans were conducted with cancer patients, using i.v. injections which are not relevant to oral administration.

Ames et al. (1981) determined the plasma and urine concentrations of amygdalin, whole-blood concentration of cyanide, and thiocyanate concentrations in serum and urine of cancer patients after i.v. (4.5 g/m^2) and oral (500 mg tablet three times daily) administration of amygdalin over a period of 21 days. After i.v. administration, high concentrations of amygdalin (up to 1,400 μg/mL) but no increase in the levels of cyanide or thiocyanate were observed in the blood serum. After oral administration, amygdalin levels were much lower (< 0.5 μg/mL) compared to i.v. administration. Cyanide was detectable in blood only after oral administration (2.1 μg/mL). Excretion rates were not determined. These findings suggest that human tissues are unable to hydrolyse amygdalin distributed in the blood after i.v. infusion. Further, absorption of amygdalin from the gastrointestinal tract appears to be marginal, but extensive degradation to cyanide occurs in the gut by the microflora.

In support of the capability of the human faecal flora to degrade amygdalin to cyanide, the release of cyanide was observed when several common human faecal bacteria were incubated with amygdalin *in vitro*. The highest activity was observed for *Bacteroides fragilis*, a constituent of human faecal microflora (Newton et al., 1981).

In a healthy human volunteer ingesting 3, 6 or 12 mg of KCN, maximum blood levels of cyanide were reached after 10–20 min and amounted to 1, 6 and 21 μM; the elimination half-life in blood was about 20 min (Schulz, 1984). In other studies on humans, half-lives of 0.34–1.0 h have been reported (Ansell and Lewis, 1970; Hartung, 1982).

Because of the high binding of cyanide to methaemoglobin in erythrocytes, the volume of distribution of cyanide depends on the methaemoglobin content of the blood and also on the total concentration of cyanide in the body (Schulz et al., 1982). As 1 g of methaemoglobin can bind approximately 60 μM of cyanide (Schulz, 1984), 1 L of blood with a normal physiological methaemoglobin level of 0.25 to 1% is able to bind 25–100 μM of cyanide. The volume of distribution of low doses of cyanide in humans has been estimated as approximately 0.075 L/kg (Schulz et al., 1982).

In contrast to cyanide, the major metabolite thiocyanate does not bind to methaemoglobin in blood and is not readily distributed into tissues but excreted in the urine. The apparent distribution volume of thiocyanate in healthy humans has a mean value of 0.25 L/kg and the half-life in blood serum ranges from 1.5 to 5 days (Schulz et al., 1979).

Abraham et al. (2016) studied the bioavailability of cyanide from foods (persipan in two different amounts, linseed, cassava or bitter apricot kernels) containing various CNGs in a five-way crossover study with at least 2 weeks between the different arms of the study. Each of 12 healthy non-smoking adults (aged 30–56 years, five females with a mean body weight of 64.6 kg and seven males with a mean body weight of 81.0 kg) ingested six medium-sized kernels (about 2.1 g, containing 6.8 mg total cyanide). The aim was for 'worst case conditions', which involved an overnight fast before the study, thorough chewing to facilitate maximal release of free cyanide from the glycosides, and consumption of 200 mL tap water after consumption and again after 60 min but no other food or water until the end of the investigation. The blood cyanide levels were determined thereafter using a GC-MS method with $K^{13}C^{15}N$ as internal standard. The maximum blood level was observed 20 min after ingestion of the apricot kernels and amounted to a mean value of 15.46 μM[15] (range 7–23), followed by a steep decrease. The dose of 6.8 mg total cyanide was selected in order to result in maximum blood cyanide levels in the region of 20 μM, a level that was considered to be safe. The mean cyanide doses corresponded to 0.105 mg/kg bw in women and 0.084 mg/kg bw in men, and the peak blood cyanide levels following consumption of apricot kernels (mean ± SD, Standard Deviation) were 20.06 ± 3.35 μM in women and 12.17 ± 3.19 μM in men. These levels did not differ statistically significantly from those of the cassava study, in which the mean peak blood cyanide concentrations were 21.30 ± 6.28 μM in women and 13.84 ± 3.44 μM in men. The differences were found to be correlated with body weight and sex, but not with age. When one of the test persons ingested bitter apricot kernels, KCN or pure amygdalin alone or together with sweet almonds (to provide the hydrolytic plant enzymes) in amounts equivalent to 6.8 mg cyanide, the peak levels of cyanide in whole blood were 15.4, 20.1, 3.4 and 10.0 μM (Abraham et al., 2016). This suggests that the cyanide present in the apricot kernels is almost

[15] A value of 14.3 μM is cited in the text while the value of 15.46 μM is reported in Table 1 of Abraham et al. (2016).

completely bioavailable, while cyanide present in pure amygdalin is only partly released upon ingestion in the absence of the hydrolytic plant enzymes.

3.1.1.3. Biomarkers

Cyanide in blood is frequently used as an exposure biomarker for cyanide poisoning. Methods applied include colorimetric reaction followed by spectrophotometric detection as well as high performance liquid chromatography with mass spectrometry (HPLC-MS), gas chromatography-microwave-induced plasma atomic emission detection (GC-MPD), gas chromatography-electronic capture detection (GC-ECD) and gas chromatrography-mass spectrometry (GC-MS) (summarised in ATSDR, 2006). In the literature, there are different opinions concerning the biomaterial (whole blood, erythrocytes or plasma) to be preferred for this purpose. In blood, cyanide is almost exclusively localised in erythrocytes and only a relative small proportion is transported via plasma. In the literature, erythrocyte to plasma ratios of at least 10:1 are frequently cited Rumack, 1983). These ratios were also confirmed by Lundquist et al. (1985). In this study, cyanide concentrations in whole blood, plasma and erythrocytes were compared in 10 non-smoking subjects and five smoking subjects. For the non-smokers, the mean cyanide values (± SD) were 0.13 ± 0.08 μM for whole blood, 0.24 ± 0.22 μM for erythrocytes and 0.02 ± 0.02 μM for plasma; for the smokers, the mean cyanide values (± SD) were 0.33 ± 0.12 μM for whole blood, 0.68 ± 0.20 μM for erythrocytes and 0.03 ± 0.02 μM for plasma. Thus, some authors recommend cyanide analysis in erythrocytes (McMillan and Svoboda, 1982; Sano et al., 1992) or in whole blood (summarised in Lundquist et al., 1985; ATSDR, 2006). Other authors state that quantifying cyanide in whole blood results in falsely high results caused by an artefactual formation of cyanide from plasma thiocyanate and oxyhaemoglobin. Furthermore, it has to be taken into account that HCN is a very weak acid and thus cyanide exists in the blood almost entirely as HCN, whose half-life in blood is less than 1 h. Therefore, all steps of storage, sample preparation and the analytic process itself have to be carried out with caution to minimise the risk of cyanide loss and falsely low levels.

In contrast to cyanide, its stable metabolite thiocyanate is confined to plasma. Therefore, some studies determined thiocyanate in serum or plasma as a measure of cyanide exposure. More recently, in addition to cyanide, ATCA (see Figure 4 Section 3.1.1.1) in plasma has been used as a potential biomarker (Lundquist et al., 1995; Logue, 2005, 2009; Vinnakota et al., 2012). Additionally, cyanide adducts with human serum albumin have been discussed recently from an analytical point of view to be a more stable and thus reliable biomarker for cyanide exposure as compared to cyanide in whole blood (Fasco et al., 2007, 2011). The CONTAM Panel is aware of other potential biomarkers for cyanide poisoning including thiocyanate in urine. Additionally, potential effect biomarkers have been identified in animal studies. As this assessment was confined to the exposure biomarker cyanide in blood, other potential biomarkers are not described here in detail.

3.1.2. Toxicity studies

3.1.2.1. Toxicity in laboratory animals

The overt clinical effects in experimental animals due to lethal levels of cyanide exposure are: dyspnoea, irregular shallow and gasping breathing, ataxia, tremors, retrocolic spasms, tonic spasms, loss of consciousness, convulsions and asphyxiation. Brain seems to be the organ most sensitive to acute cyanide toxicity. DECOS (2002) note that these effects occur irrespective of the route of exposure. They refer to the following studies: Ballantyne (1983, 1994); US EPA (1992); ATSDR (1997). Furthermore, the cyanide ion being the common agent that induces tissue anoxia by inhibiting tissue cytochrome oxidase activity in different species, there are no qualitative differences in acute toxicity between cyanide compounds (Way, 1984; US EPA, 1988; WHO, 2004).

Acute toxicity of cyanides

Acute oral LD_{50} values for cyanide reported in previous assessments (WHO, 1965; DECOS, 2002; ATSDR, 2006) ranged from 2.13 to 6 mg/kg bw (see Table 1).

Table 1: Summary of acute toxicity studies with cyanides after oral exposure[(a)]

Compound	Species	LD_{50} (mg/kg bw)	LD_{50} (mg CN^-/kg bw)	References
HCN	Rabbit	2.49	2.39	DECOS (2002), Ballantyne (1987)
	Rat	4.21	4.05	DECOS (2002), Ballantyne (1987)
NaCN	Rabbit	5.11	2.71	DECOS (2002), Ballantyne (1987)
	Rat	5.72	3.04	DECOS (2002), Ballantyne (1987)
	Rat	8	4.2	ATSDR (2006), Smyth et al. (1969)
KCN	Rat	10–15	4.02–6.03	WHO (1965), Conn (1979)
	Rat	7.48	2.99	DECOS (2002), Ballantyne (1987)
	Mouse	8.50	3.4	DECOS (2002), Ballantyne (1987)
	Mouse	15.8	6	WHO (2004), Ferguson (1962)
	Rabbit	5.82	2.33	DECOS (2002), Ballantyne (1987)
	Dog	5.3	2.13	WHO (1965), Conn (1979)
$Ca(CN)_2$	Rat	22	3.49	ATSDR (2006), Smyth et al. (1969)

bw: body weight; HCN: hydrocyanic acid; HPLC-DAD: high performance liquid chromatography with diode-array detectionl; LD_{50}: medium lethal dose.
(a): Medium lethal oral doses (LD_{50}) for cyanides obtained from previous assessments (WHO, 1965; DECOS, 2002; ATSDR, 2006) and based respectively on the works of Conn (1979), Ballantyne (1987) and Smyth et al. (1969).

Short-term toxicity of cyanides

Kreutler et al. (1978) (reviewed by US EPA, 2010). *Experimental design:* Two-week toxicity study, male weanling albino rats fed diets containing either low protein (2% casein) or normal protein (20% casein) and supplemented with 0.2% (2 g KCN/kg). *Evaluations:* Body and thyroid weight and plasma thyroid stimulating hormone (TSH). *Negative results:* No difference in body weight among cyanide and respective control group, no effect on serum TSH or thyroid weight was observed in rats subjected to KCN in a normal-protein diet. *Effects of KCN:* Increase in thyroid weight and TSH serum levels in rats subjected to KCN in a low-protein diet.

Tulsawani et al. (2005). *Experimental design:* Female Wistar rats dosed by gavage to 7 mg KCN/kg bw once daily for 14 days. *Evaluations:* Organ-body index, haematological, clinical chemical and urine analyses, histopathological examination. *Negative results:* Although this dose falls in the LD_{50} range identified for KCN (see Table 1), the authors failed to detect any mortality in the treatment group. As KCN solutions were prepared freshly and administered by gavage, instability of cyanide concentration in solution and/or detoxification mechanisms does not provide reasons for these results. No significant changes in body weight and organ-body index. *Effects and altered biomarkers due to KCN:* Increased serum thiocyanate and blood glucose, decreased alanine aminotransferase (ALT). Significant inhibition of cytochrome *c* activity, signs of lipid peroxidation and impairment of antioxidant defence specifically in the brain, as well as demyelination in *medulla oblongata*, chromatolysis and degeneration of cerebrocortical cells. Fatty changes of various grades and vacuolar degeneration of hepatocytes, varying in size and more pronounced in the central region, coupled to eosinophilic debris. Hepatic rhodanese activity was also significantly decreased. In addition, focal myocardial degeneration glomerular congestion, tubular lesions displaying vacuolar degeneration at proximal tubular epithelial cells and scattered tubule disorganisation due to damaged renal parenchyma were also observed.

Sousa et al. (2002) (reviewed by US EPA 2010). *Experimental design:* Fifteen-day toxicity study, male adult Wistar rats treated with KCN in drinking water adjusting KCN concentration to body weight and water consumption in order to administer 0, 0.3, 0.9, 3.0 or 9.0 mg/kg per day. *Evaluation:* Haematological, biochemical (in serum) and urine analyses, histopathology. *Negative results:* Biochemical and urine analysis revealed no or only sporadic changes which were not dose related. *Effects and altered biomarkers due to KCN:* Increased plasma thiocyanate levels in all experimental groups. 'Moderate' to 'severe' congestion and cytoplasmic vacuolisation of the epithelial cells of the kidney proximal tubules at 3.0 or 9.0 mg/kg per day (both severe), hydropic degeneration of hepatocytes at 9.0 mg/kg per day (severe), resorption vacuoles in the thyroid gland of animals in all groups, including controls (minimal), which increased in severity up to 0.9 mg/kg day (severe). Lowest observed adverse effect level (*LOAEL*)–No observed adverse effect level (*NOAEL*): Although resorption vacuoles in the thyroid gland were reported as moderate at 0.3 mg KCN/kg (0.12 mg/kg CN^-) and severe at 0.9 mg KCN/kg, based on moderate kidney vacuolisation and congestion, the US EPA (2010)

identified a NOAEL of 0.9 mg KCN/kg per day (0.36 mg/kg CN^-) and a LOAEL of 3 mg/kg per day (1.2 mg/kg CN^-) from this study.

Palmer and Olson (1979) US EPA (2010). *Experimental design:* Male Sprague-Dawley rats treated with KCN 200 mg/L in drinking water or food for 21 days. *Evaluations:* Body and liver weight. *Effects due to KCN:* A statistically significant 17% increase in absolute liver weight. The dietary part of this study has been considered inconsistent due to the instability of cyanide in food.

EFSA (2004) (based on the revision of original data by Leuschner and Neumann, 1989; NOAEL and LOAEL not provided). *Experimental design:* Thirteen-week toxicity study, male Sprague-Dawley rats administered KCN in the drinking water (40, 80 and 160/140 mg KCN/kg bw per day). *Evaluation:* Behaviour, external appearance, body weight, haematological, clinical chemical (in serum) and urine analyses, histopathological examination (brain, kidneys, heart, liver and testes plus thyroid in the high-dose group (160/140 mg) KCN/kg because of observed reduced body weight gain, reduced water consumption and mortality). *Negative results:* No histopathological damage. Decreased body weight and food consumption in KCN rats caused by a decrease in water consumption due to a decreased palatability. *Effects due to KCN:* Dose-related higher levels of protein in urine in KCN group compared to controls. Rats treated with 40 and 80 mg/kg bw KCN exhibited a slight increase in relative organ weight, which became clearly increased at 160 and 140 mg/kg while thymus weight was reduced in the high-dose group. No histopathological damage. Decreased body weight and food consumption in KCN rats caused by a decrease in water consumption due to a decreased palatability.

NTP (1993) (reviewed by US EPA, 2010). *Experimental design:* Thirteen-week study, male and female F344/N rats and B6C3F1 mice received 0, 3, 10, 30, 100 or 300 mg NaCN/L per day in drinking water. The average exposures were 0, 0.3, 0.9, 2.7, 8.5 and 23.6 mg NaCN/kg bw per day in male rats and 0, 0.3, 1.0, 3.2, 9.2 and 23.5 mg NaCN/kg bw per day in female rats. Average exposures in mice were 0.5, 1.8, 5.1, 16.2 and 45.9 mg NaCN/kg bw per day in males and 0.6, 2.1, 6.2, 19.1 and 54.3 mg NaCN/kg bw per day in females, respectively. *Evaluation:* Behaviour, external appearance, body weight, haematological, clinical chemical (in serum) and urine analyses, histopathological examination (complete), sperm motility and vaginal cytology. *Negative results:* No deaths, clinically significant body weight-, histopathologic-, or clinical pathology changes were evident in both sexes of either species. Haematological, clinical chemistry and urinalysis evaluations of rats and mice revealed minimal changes that were considered not biologically significant. *Effects and altered biomarkers due to NaCN:* reduced water consumption (10–30%) in rats and mice in the 100 and 300 mg NaCN/L per day groups. Urine thiocyanate (monitored only in male rats) increasing with dose starting from 10 mg NaCN/L (0.9 mg NaCN/kg bw per day). Adverse effects on rat reproductive system observed were a slight dose-dependent reduction in cauda epididymal weight in all groups of exposed male rats (only rats exposed to $\geq$ 30 mg/L NaCN were considered) and reduced number of spermatid heads per testis at 300 mg NaCN/L. In male mice, relative weights of the epididymis and cauda epididymis were significantly decreased at 100 mg NaCN/L (16 mg NaCN/kg bw per day) (US EPA, 2010). Concentrations of 100 and 300 mg NaCN/L caused a significant increase in length of pro-oestrus and dioestrus relative to oestrus and metoestrus in female rats. *LOAEL:* Based on significantly decreased cauda epididymis weights, US EPA (2010) calculated a LOAEL of 30 mg NaCN/L per day (2.7 mg NaCN/kg per day; 1.44 mg/kg CN^-) in rats and of 100 mg/L (16 mg NaCN/kg bw per day; 8.51 mg/kg CN^-) in mice.

Philbrick et al. (1979). *Experimental design:* Eleven-month toxicity study, first evaluation at 4 months, 1,500 KCN mg/kg feed in male weanling rats (average weight 43 g) fed two different diets containing 10% casein with different supplementation with DL-methionine, potassium iodide and vitamin B_{12} for 11 months. *Evaluations*: Behaviour, external appearance, body weight, plasma thyroxine, thyroxine secretion rates and urinary thiocyanate. *Negative results:* No death or clinical signs. *Effects due to KCN:* Body weight significantly lower starting from 8 weeks of treatment, plasma thyroxine and thyroxine secretion rate significantly reduced at 4 months of treatment irrespective of diet, suggesting depressed thyroid gland functions.

Conclusions on acute and short-term toxicity studies with cyanides

The acute oral LD_{50} values for cyanide range from 2.13 to 6 mg/kg bw. The NTP (1993) 13-week study provided a LOAEL of 2.7 mg NaCN/kg bw per day (1.44 mg/kg CN^-) in rats and of 16 mg NaCN/kg bw per day (8.51 mg/kg CN^-) in mice, based on decreased cauda epididymis weights. The LOAEL derived in rats was confirmed by a 15-day study (1.2 mg/kg CN^-) by Sousa et al. (2002), which also provided a NOAEL of 0.9 mg KCN/kg bw per day (0.36 mg/kg CN^-) based on histological changes in the kidneys. In general, histopathological changes were observed after 2 weeks of

repeated exposure to cyanide, but were absent in sub-chronic studies where organs weights were the parameters most frequently affected in adult rodents. No clinical signs were observed in short-term studies and changes in haematology and urine were minor, sporadic and not dose related. In none of the reported short-term studies was mortality observed at doses up to 40 mg CN^-/kg bw per day, even though some of the doses were equal to or higher than the oral LD_{50} for cyanide. Since in the short-term studies analysed, cyanide was administered through the diet or drinking water, absence of mortality is possibly due to a slower absorption rate following dietary exposure, thus not exhausting the detoxification capacity of the enzyme rhodanese, which occurs after bolus administration in LD_{50} tests (Hayes, 1967; US EPA, 2010).

Acute and short-term toxicity of amygdalin

Acute toxicity of amygdalin

Toxicity of CNGs depends on HCN release by hydrolysis of gut microbiota and subsequent absorption. Accordingly, a single oral dose of 600 mg amygdalin (35.4 mg HCN per kg bw) induced blood cyanide increases (2.6–4.5 μg/mL) and mortality in conventional but not germfree rats of the same strain (Carter et al., 1980; FAO/WHO, 2012). The estimated LD_{50} for the amygdalin in rats ranges from 522 mg (30.8 mg HCN) to 880 mg (51.9 mg HCN)/kg bw (Adewusi and Oke, 1985; Newton et al., 1985). This lower acute toxicity as compared to HCN is explained by the slow and incomplete release of HCN from CNGs (EFSA, 2004).

Short-term toxicity of amygdalin

Oyewole and Olayinka (2009). *Experimental design:* Fourteen-day toxicity study, 20 mg amygdalin/kg bw (equivalent to 1.18 mg HCN; obtained from Sigma[16]) orally administered by cannula to male Wistar rats daily. *Evaluations:* Haematological and serum analysis, histological examination of the liver. *Effects and altered biomarkers due to amygdalin:* Mortality (one out of eight rats, 12.5%), significant increase in blood cyanide (15.23 ± 1.22 μmol/L), haemoglobin concentration, packed cell volume and serum lactate and a decrease in whole blood pH.

Basu (1983) (reviewed by EFSA, 2004). *Experimental design:* Twenty-four day toxicity study, guinea pigs (Duncan-Hartley) received daily 10 mg laetrile (amygdalin, obtained from Sigma[15]; equivalent to 0.59 mg HCN) dissolved in 10% sucrose solution with and without ascorbic acid (100 mg). *Negative results:* Administration of laetrile without or with ascorbic acid had no significant effect on body and liver weight. *Alterations of biomarkers due to amygdalin:* Treatment with laetrile alone for 4, 16 and 24 days resulted in a significant increase in urinary levels of thiocyanate.

Conclusions on acute and short-term toxicity of amygdalin

The available data do not allow identification of NOAELs and LOAELs.

Developmental studies with cyanide and amygdalin

de Sousa (2007). *Experimental design:* Pregnant rats received KCN at different concentrations in drinking water, from gestation day (GD) 6 to GD 20, to obtain the target doses of 1.0, 3.0 and 30.0 mg/kg bw per day of KCN. *Evaluations:* Biochemical analyses and histopathology in the dams (GD 20 and at weaning) and litter at postnatal day (PND)21 plus examination for developmental malformations in foetuses GD 20. *Negative results:* No death; body weight, reproductive data and skeletal variation were unaffected. *Effects due to KCN:* Treatment resulted in a significant and dose-dependent increase in serum levels of thiocyanate only in dams sacrificed at GD 20. A significant increase in serum glucose levels was also evident, but only at 30 mg/kg per day KCN. Hepatic and central nervous system congestion and gliosis were moderate in both dams and litter at postnatal day (PND)21 at the highest dose of 30 mg KCN/kg per day. A dose-dependent increase in the number of resorption vacuoles at the thyroid occurred in dams: mild for 1, moderate for 3 and severe for 30 mg KCN/kg per day. As this effect is not associated with alteration to cholesterol levels (a parameter indicative of thyroid functions), the authors concluded that the altered thyroid histology is a reflection of the compensatory increase in the efficacy of thyroid hormone synthesis. A NOAEL of 3 mg KCN/kg (1.2 mg/kg CN^-) and LOAEL of 30 mg KCN/kg (12 mg/kg CN^-) were derived on the basis of histological alterations both in dams and PND 21 litters.

[16] Sigma uses the term laetrile as a synonym for amygdalin which is not correct.

Willhite (1982). *Experimental design:* Female Golden Syrian hamsters (strain LVG) were treated by gavage with a single dose of D,L-amygdalin (200, 225, 250 and 275 mg/kg bw or D-amygdalin (300 mg/kg bw) obtained from the Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland[17]) on GD 8. *Evaluations:* Examination for developmental malformations in foetuses at GD 14. *Effects due to amygdalin:* Hyperpnoea, dyspnoea, ataxia and tremors occur in mothers exposed to ≥ 250 mg/kg bw (equivalent to 14.2 mg CN^-/kg bw). The oral dose of 300 mg D-amygdalin/kg caused identical malformations at similar incidences. One mother exposed to D,L-amygdalin 275 mg/kg and two mothers exposed to D-amygdalin 300 mg/kg died. Percentages of abnormal foetuses progressively increase with the dose. In particular, exencephaly, encephalocoele and skeletal malformations resulted at doses of ≥ 250 mg/kg bw (≥ 14.2 mg CN^-/kg bw). At 200 mg D,L-amygdalin/kg bw (11.3 mg CN^-/kg bw), fused ribs were observed in two offspring of one mother (maternal toxicity not reported). A supplementary group was treated with 300 mg D-amygdalin/kg. No teratogenic effects were noted when hamsters received D,L-amygdalin [275 mg/kg bw (15.5 mg CN^-/kg bw)] intravenously. The teratogenic effects found were considered to be due to cyanide released by bacterial β-glucosidase in the gastrointestinal tract.

A NOAEL of 225 mg D,L-amygdalin/kg bw (12.8 mg CN^-/kg bw) and a LOAEL of 250 mg D,L-amygdalin/kg bw (14.2 mg CN^-/kg bw) is derived on the basis of foetal abnormalities.

Conclusions on developmental studies with cyanide and amygdalin

Indications of teratogenicity in offspring of hamsters treated with ≥ 250 mg/kg bw amygdalin (equivalent to ≥ 14.2 mg CN^-/kg bw) on day 8 of gestation were observed in one study while signs of toxicity were revealed both in rat dams and their weanling offspring after exposure to 30 mg KCN/kg (12 mg CN^-/kg). A NOAEL of 225 mg D,L-amygdalin/kg bw (12.8 mg CN^-/kg bw) and a LOAEL of 250 mg D,L-amygdalin/kg bw (14.2 mg CN^-/kg bw) can be derived based on foetal abnormalities. A NOAEL of 3 mg KCN/kg (1.2 mg/kg CN^-) and LOAEL of 30 mg KCN/kg (12 mg/kg CN^-) can be derived based on histological alterations both in the dams and PND 21 litters.

3.1.2.2. Humans

The clinical signs of cyanide toxicity include headache, dizziness, mental confusion, stupor, cyanosis with twitching and convulsions, followed by terminal coma. The EFSA AFC Panel noted that the human acute lethal oral dose of HCN is reported to be 0.5–3.5 mg/kg bw. Cyanogenic glycosides are hydrolysed by the gut microflora to slowly and incompletely release cyanide with subsequent absorption and therefore the acute toxicity for CNGs would be lower than that of HCN. Well-nourished individuals have ingested 1,000 mg (equivalent to 59 mg HCN) or more of pure amygdalin every day without any evidence of 'side effects' (EFSA, 2004).

It is frequently cited that the toxicity threshold for cyanide in whole blood is about 20 μM. This originates mainly from Rumack (1983) who summarised blood cyanide concentrations and associated symptoms, although noting that they were 'very questionable'. The ranges were no signs of toxicity at blood cyanide concentrations of 0.2–0.5 mg/L (8–20 μM); flushing and tachycardia occur at 0.5–1.0 mg/L (20–38 μM), a reduced level of consciousness (obtunded) at 1.0–2.5 mg/L (48–95 μM), coma at 2.5–3.0 mg/L (95–114 μM) and death at 3.0 mg/L (114 μM). Rumack (1983) noted that a very wide range of lethal concentrations had been reported (0.1–230 mg/L; 4–8,570 μM), and that in many cases blood was sampled at autopsy without taking into account the time since exposure. In addition, cyanide concentrations decrease in stored blood, which could further contribute to falsely low values being reported (Hall and Rumack, 1986). The basis for the ranges cited by Rumack, and whether they incorporated some form of extrapolation to allow for the rapid elimination of cyanide, is unclear. Furthermore, no information is available on the extent to which these ranges cover the full range of possible variability in sensitivity to cyanide within different subgroups, such as children. Some support for assuming the threshold of 20 μM comes from Schulz et al. (1982), who reported that, based on a review of the literature, biochemically detectable disturbances occur at erythrocyte cyanide concentrations above 40 μM, clinically recognisable symptoms above 200 μM and possible fatalities above 400 μM. As the ratio of cyanide concentrations in erythrocytes to that in plasma is at least 10:1 (Rumack, 1983), Abraham et al. (2016) concluded that the concentration of 200 μM in erythrocytes approximately corresponds to 20 μM in whole blood. However, the CONTAM Panel noted that there is uncertainty in the applied (10:1) erythrocyte to plasma ratio of cyanide. Some of the evidence for the toxicity threshold of 20 μM derives from clinical studies. For example, Aitken et al. (1977) monitored

[17] Amygdalin is present in apricot kernels as the D-stereoisomer and not as a mixture of the D and L forms.

eight female and five male patients (aged 13–66) receiving the hypotensive agent sodium nitroprusside (which contains 44% cyanide) undergoing surgery for repair of an intracranial aneurysm. They found that blood cyanide levels above 0.53 mg/L (20 μM) were associated with proportionately greater degrees of metabolic acidosis.

The AFC opinion summarised a number of case reports of human poisoning associated with ingestion of amygdalin preparations or apricot kernels.[18] For amygdalin preparations, these included a 11-month-old girl who 'accidentally' ingested –one to five amygdalin tablets (500 mg, equivalent to 28.5 mg CN^-), and a 17-year-old girl suffering from cancer who regularly took four ampoules of laetrile (3 g amygdalin, equivalent to 171 mg CN^-) intravenously who became ill after swallowing orally the content of 3.5 ampoules of laetrile. For apricot kernels, these included nine cases of cyanide intoxication of children who had probably ingested more than 10 wild apricot kernels (208 mg CN^-/100 g).

The CONTAM Panel identified two additional case reports with information on the number of kernels consumed that were published before 2004 and not included in the AFC Panel opinion, but were cited in other risk assessments. A 49-year-old female was hospitalised following the consumption of 20–40 apricot kernels (Rubino and Davidoff, 1979a,b). The authors considered that this amount would have been lethal had the woman not vomited substantial quantities of chewed kernels. A 41-year-old woman was found in a comatose and hypothermic state following the consumption of approximately 30 bitter apricot kernels and recovered following administration of a cyanide antidote. Her blood cyanide level was 43.1 μM (1.16 mg/L) 5 h after ingesting the kernels (Suchard et al., 1998).

Since the AFC opinion of 2004, a number of additional case reports have been published.

Amygdalin and amygdalin-containing herbal preparations

A 68-year-old woman with carcinoma of the bladder began to feel ill about 30 min after taking six 500 mg amygdalin tablets (reported to correspond to 100 mg of cyanide) and presented at hospital about 2 h later with signs of cyanide toxicity. An antidote was administered and she made a full recovery. The authors noted that the severity of her reaction was not expected from the amygdalin alone and postulated that the effects of cyanide were exacerbated by coingestion of megadoses of vitamin C (4,800 mg) (Bromley et al., 2005).

A 51-year-old woman with a history of breast cancer developed symptoms of cyanide toxicity after inadvertently taking five 500 mg laetrile tablets and recovered following administration of a cyanide antidote (Martinelli et al., 2008).

A 63-year-old woman with a history of metastatic carcinoma of the lung was diagnosed with cyanide poisoning. She had been taking a herbal product containing amygdalin extract (500 mg) over 5 months. Her blood cyanide concentration was 4.38 mg/L (162 μM). She recovered after antidote treatment (Lam et al., 2012).

A 4-year-old boy with a history of malignant brain disease was diagnosed with cyanide poisoning. He had been given a number of complementary medicines, including a preparation referred to as 'vitamin B17' (4 × 500 mg/day) and apricot kernels (5–10/day). His serum cyanide level was 0.5 mg/L (18.5 μM) 2 h after the last administration of the 'vitamin B17' preparation. He recovered after administration of a cyanide antidote (Sauer et al., 2015). The CONTAM Panel noted that the concentration has been measured in serum and that the corresponding concentration in whole blood level would have been an order of magnitude higher (see also Section 3.1.1).

Apricot kernels

There are a number of reports of cyanide toxicity in children who have ingested apricot kernels (sometimes referred to as apricot seeds in the individual reports). According to National Poison Centre data, there were 260 cases of paediatric apricot kernel poisoning in Turkey during 2000–2004 (Oto Geçim et al., 2006; cited by Akyildiz et al., 2010). Akyildiz et al. (2010) reviewed admissions of children to a hospital in Turkey with signs of cyanide toxicity following ingestion of apricot kernels. Thirteen children aged 3–9 years (four male, nine female) were admitted between 2005 and 2009. The median (range) of apricot kernels consumed was 8 (5–21). All patients recovered following treatment.

A 28-month-old girl suffered severe cyanide toxicity after eating approximately 10 apricot kernels, and died 22 days later despite antidote treatment. Her blood cyanide level was more than 3 mg/L (110 μM) 20 h after arrival at the emergency department (Sahin, 2011).

[18] Apricot kernels are sometimes referred to as apricot seeds in the individual reports, but the term kernels is used in this opinion.

A previously healthy boy aged 27 months (11 kg) was diagnosed with cyanide toxicity after eating 'plenty of apricot pits' and recovered after treatment (Kaya et al., 2012).

A 58-year-old man with metastatic colon carcinoma was found to have elevated serum liver enzymes when reporting to hospital for chemotherapy. He had been taking 70 apricot kernels (chopped thoroughly) per day for 45 days up to a week before the outpatient visit. He did not have any of the common side effects of amygdalin use, and no lactic acidosis. A blood thiocyanate level of 71 mg/L was measured, which the authors back-extrapolated to a concentration of 118 mg/L on the last day of apricot kernel consumption (Seghers et al., 2013).

Almış et al. (2014) reviewed admissions to a paediatric emergency service in Turkey due to plant poisoning between January 2010 and May 2012. Five of the 23 cases were associated with consumption of apricot kernels, but no further detail is available on these patients.

In the bioavailability study of Abraham et al. (2016), described in Section 3.1.4, the dose level was selected in order to result in maximum blood cyanide levels in the region of 20 μM, because this was considered to be a safe level citing Rumack (1983) and Schulz et al. (1982). The average doses corresponded to 0.105 mg/kg bw in the women and 0.084 mg/kg bw in men. The volunteers did not report any symptoms of cyanide toxicity; however, clinical parameters such as blood lactic acid were not measured. The highest blood level of 31.9 μM was found in the woman with the lowest body weight (60 kg) when she consumed cassava: the cyanide dose was equivalent to 0.113 mg/kg bw. In a separate study, one of the 12 volunteers, an 80-kg male, consumed persipan paste in amounts corresponding to 6.8, 13.6 and 27.2 mg cyanide (equivalent to 0.085, 0.17 and 0.34 mg/kg bw). Again he did not report symptoms of cyanide toxicity; however, his maximum blood cyanide level did not exceed 20 μM.

In conclusion, cases of acute cyanide toxicity have resulted from ingestion of amygdalin preparations and of bitter apricot kernels, and some of these cases were fatal. For adults, the number of kernels consumed was 20 or more. For children five or more kernels appear to be toxic. In a bioavailability study in a small number of healthy adults, the subjects did not report symptoms of cyanide toxicity at total cyanide doses of up to 0.113 mg/kg bw in women and 0.34 mg/kg bw in men (the highest doses tested), but there were no clinical measures of toxicity.

3.1.3. Mode of action for acute toxicity

The primary mode of action underlying acute toxicity of cyanide involves impairment of oxidative phosphorylation, a process whereby oxygen is utilised for the production of essential cellular energy sources in the form of adenosine triphosphate (ATP). An essential part of this process includes the transfer of electrons from nicotinamide adenine dinucleotide (NADH), to oxygen through a series of electron carriers. This is catalysed by the cytochrome oxidase enzyme system in mitochondria (Beasley and Glass, 1998). Cyanide inhibits the terminal enzyme in this chain reaction, namely cytochrome oxidase a3, which is necessary for the reduction of oxygen to water in the fourth complex of oxidative phosphorylation (Hamel, 2011). More specifically, cyanide binds to ferric iron (Fe^{3+}) in the haem moiety of the oxidised form of cytochrome oxidase a3 to suppress its oxidative function and thereby to block cells from using oxygen, which is the substrate of normal cellular respiration (Hall and Rumack, 1986; Beasley and Glass, 1998; Guidotti, 2006; Hamel, 2011; Sahin, 2011). Consequently, tissue utilisation of oxygen is inhibited with rapid cessation of aerobic metabolism and impairment of vital functions. Other metabolic processes continue and the rate of glycolysis markedly increased leading to accumulation of intracellular NADH (Veech et al., 1970). Furthermore, produced pyruvate can no longer be utilised *via* the impaired Krebs cycle and is reduced to lactate, resulting in a state of metabolic acidosis (Beasley and Glass, 1998). Glucose is also partly converted by the pentose phosphate pathway leading as well to an increase in lactic acid with a decrease in ATP/ adenosin diphosphate (ADP) ratio (Way, 1984). Overall, cyanide causes cells to switch from an aerobic (oxygen-dependent) metabolism mode that yields ATP to anaerobic (oxygen-independent) energy production, which generates toxic
by-products, such as lactate (Hall and Rumack, 1986; Guidotti, 2006; Hamel, 2011; Sahin, 2011). Characteristics features are thus hypoxia and acidosis.

The heart and brain are particularly prone to cyanide acute toxicity, because of their requirement for a continuous supply of oxygen and ATP generated from aerobic metabolism (Guidotti, 2006). Poor oxygen extraction associated with cessation of aerobic cellular respiration equally leads to an accumulation of oxygen in the venous supply indicated by blood gas analysis and a reduced

arteriovenous oxygen saturation difference. Some cyanide also binds to methaemoglobin, the ferric form of haemoglobin, which accounts for normally 1–2% of all haemoglobin.

It should also be mentioned that cytochrome oxidase a3 is not the only enzyme negatively affected by cyanide and that additional mechanisms may drive its acute toxicity (Beasley and Glass, 1998). In this respect, cyanide inhibits about 40 enzymes, including a number of other important metalloenzymes containing iron, copper or molybdenum ions, such as alkaline phosphatase, carbonic anhydrase, catalase, peroxidase, ascorbic acid oxidase, xanthine oxidase and succinic dehydrogenase. All these reactions may contribute to the toxicity profile of cyanide as well (WHO, 2004) leading to cellular signalling unbalance and the occurrence of centrally mediated symptoms (i.e. tremors), cardiac and peripheral autonomic responses.

3.1.4. Derivation of an acute reference dose

Cyanide is released from CNGs (amygdalin) in apricot kernels during chewing and as a result of metabolism in the gastrointestinal tract. This process has resulted in cases of cyanide poisoning, including fatalities following ingestion of apricot kernels. Therefore, the CONTAM Panel concluded that an ARfD should be established for cyanide in order to consider the acute health risks related to CNGs in apricot kernels.

The oral LD_{50} of cyanide in various animal species is in the region of 2.13–6 mg CN^-/kg bw. In humans, an acute lethal dose of 0.5–3.9 mg/kg bw is reported. These data suggest that there is no major difference in species sensitivity, but do not provide a suitable basis for derivation of an ARfD because of the uncertainty regarding the steepness of the dose–response relationship and extrapolation from the lethal dose to a NOAEL. The lowest NOAEL and LOAEL from the animal toxicity data reviewed are 0.36 and 1.2 mg CN^-/kg bw, respectively, from a study in rats exposed to KCN in their drinking water for 15 days. No mortalities were reported in repeat dose toxicity studies in rodents at doses up to 40 mg/kg bw per day, even though some of the doses clearly exceeded the LD_{50}. Most of these studies involved administration of cyanide salts in drinking water, which would be consumed throughout the day and night resulting in lower peak cyanide concentrations in blood than with bolus administration. Thus these studies are of questionable relevance to the acute toxicity of cyanide.

Therefore, despite their limitations, the human data are the preferred basis for deriving an ARfD in order to avoid the uncertainty in species extrapolation. The reports of cyanide poisoning following ingestion of apricot kernels do not provide sufficient information on the numbers of kernels consumed and their CNG content, to allow estimation of cyanide exposure. The CONTAM Panel concluded that the blood cyanide concentration of 20 μM, which is reported to be a threshold for toxicity (Rumack, 1983), should be extrapolated to an external dose of cyanide to provide the basis for the ARfD.

In the bioavailability study of Abraham et al. (2016), 12 healthy adult volunteers consumed CNGs, corresponding to 6.8 mg total cyanide, from a number of different sources including bitter apricot kernels and cassava. The amount of CNGs was selected with the aim of producing a peak blood cyanide concentration of 20 μM in the volunteers as this was considered to be a safe level. The highest blood level of 31.9 μM was found in the woman with the lowest body weight (60 kg), who received the highest cyanide dose expressed on a body weight basis (0.113 mg/kg bw) when she consumed cassava. This single dose was used by Abraham et al. (2016) in proposing an ARfD. The CONTAM Panel noted that the peak blood cyanide concentration of this individual was much higher than that of the other volunteers when she consumed cassava, but not when she consumed bitter apricot kernels, and there was no explanation for this difference. Therefore, the CONTAM Panel concluded that the mean data should be used in deriving the ARfD, rather than the data from this individual. The mean cyanide dose was 0.105 mg/kg bw in the women, which resulted in a mean peak blood concentration of approximately 20 μM following consumption of both bitter apricot kernels and cassava. In men, the mean cyanide dose was 0.084 mg/kg bw and the mean peak blood cyanide concentration was lower than 20 μM.

Abraham et al. (2016) noted that cyanide is distributed in the body to the extent of about 40% in blood, which has a volume of about 0.090 L/kg bw. Using these assumptions, and assuming 100% bioavailability of 6.8 mg cyanide from bitter apricot kernels and cassava, the peak blood concentration would be 0.47 mg/L (17 μM). Considering that following consumption of apricot kernels and cassava in the Abraham et al. (2016) study, the mean peak blood concentration was 20–21 μM in females and 12–14 μM in males, it appears that the bioavailability was lower in males than in females, and this is not just due to the differences in body weight. Therefore, in order to be conservative, the CONTAM

Panel concluded that the cyanide dose of 0.105 mg/kg bw resulting in the blood concentration of about 20 μM in the females should be considered as a NOAEL and used as the basis for the ARfD.

An UF of 10 is commonly used in deriving a health-based guidance value from a NOAEL in a human study, comprising equal subfactors of 3.16 for interindividual variation in toxicokinetics and toxicodynamics (EFSA Scientific Committee, 2012). Assuming that CNGs are completely broken down to cyanide, and that the cyanide is rapidly absorbed and 100% bioavailable, it is highly unlikely that individual variability could lead to appreciably higher peak blood levels. Because of the very short time to peak blood level, individual differences in metabolism (e.g. due to reduced activity of rhodanese), could delay elimination, but are unlikely to have an impact on the peak level. The variations in peak blood levels seen in the study from Abraham et al. (2016) were small. Women have a smaller distribution volume of blood than men and children have a larger blood volume (per kg/bw) than adults. Therefore, the CONTAM Panel concluded that a default factor of 3.16 was not required and that a factor of 1.5 was sufficient to cover any additional variability in toxicokinetics.

The CONTAM Panel noted the lack of information on whether potentially sensitive individuals (e.g. children) were included in the database underpinning the assumption that a blood cyanide level of 20 μM is a toxicity threshold, and that the study of Abraham et al. (2016) was conducted in a small number of healthy volunteers. The CONTAM Panel concluded that a toxicodynamic subfactor should be applied, and in the absence of cyanide-specific data on individual sensitivity, adopted the default subfactor of 3.16. Dividing the NOAEL of 0.105 mg/kg bw by the factors of 1.5 and 3.16, with rounding to a single significant figure, an ARfD for cyanide of 0.02 mg/kg bw (20 μg/kg bw) is established for use in assessing the risks associated with the presence of CNGs in apricot kernels.

3.2. Consumption data

The promoted portion sizes as provided by 11 websites promoting the consumption of raw apricot kernels ranged from 5 up to 10 kernels per day for the general population, while for cancer patients, the promoted portion size reached 60 apricot kernels per day.

3.3. Previously reported occurrence data

The Panel noted that the amount of amygdalin, and therefore of cyanide (originating from amygdalin) in apricot kernels, can vary widely. Bitter apricot cultivars are reported to contain significantly more amygdalin compared to the sweet cultivar (Karsavuran et al., 2014). Yildirim and Askin (2010) found that the concentration of amygdalin in bitter kernels was higher than that in the sweet cultivar, while Femenia et al. (1995) reported that sweet apricot kernels (without skin) do not contain any amygdalin.

Other authors refer that significant differences in the content of amygdalin, and consequently of HCN, in apricot kernels are due to geographical and climate characteristics. Chaouali et al. (2013) noted that a dry climate and intense sunlight promote cyanogenesis, and that differences in amygdalin concentration may be also ascribable to different agricultural practices. Finally, it has been reported that the content of amygdalin may also vary according to the age of the plant at harvest time: amygdalin gradually increases during plant growth, reaching the highest concentrations at maturity (Chaouali et al., 2013).

Most of the information available from the scientific literature is related to amygdalin content in apricot kernels; thus, for the purpose of comparison, levels of HCN have been estimated by using the conversion factor of 0.059 (as defined by JECFA, see Section 1.3.3). Since, as stated earlier, HCN exists in aqueous biological systems always in a mixture of its undissociated and dissociated forms, the respective levels are named 'cyanide concentration' and are expressed in mg/g (Table 3). Concentration data of cyanide (originating from amygdalin) in bitter or sweet apricot kernels obtained from eight scientific studies are summarised in Table 2. Cyanide concentrations ranged from a minimum of 0.5 to a maximum of 3.8 mg/g in apricot kernels described as bitter, while a range of 0–1.7 mg/g was identified for apricot kernels described as sweet. Mean kernel weights of 0.4–0.6 g were reported in these studies. However, there is considerable variation in the weights of individual kernels. Abraham et al. (2016) weighed all kernels (n = 1,334) in a 500-g pack of bitter apricot kernels, which had a mean ± SD of 0.37 ± 0.1 g (median 0.36 g, range 0.12–0.84 g).

Table 2: Cyanide concentration levels in apricot kernels reported in the literature and additional information (methodology used, kernel characteristics when analysed and kernel weights)

Cultivar	Cyanide concentration (mg/g)			Methodology used to measure amygdalin or HCN in kernels	Kernels as analysed	Mean kernel weight (g)	References
	Mean	Min	Max				
Reported as bitter	–	2.7[a]	3.8[a]	Acidic titration method (AOAC, 1980)	Dry matter, without skin	0.5	Femenia et al. (1995)
	–	1.5	1.7	Alkaline-titration method (AOAC, 1995)	–	–	Gupta and Sharma (2009)
	3.3[a]	2.6[b]	3.8[a]	HPLC-DAD	Dry matter	–	Yildirim and Askin (2010)
	0.8	0.5	1.2	Argentometric method, according to ISO 2164-1975 standard, relating to the dosage of CNGs in leguminous plants)	Dry matter	–	Chaouali et al. (2013)
	1.5[a]	0.8[a]	2.4[a]	HPLC-DAD	Dry matter	–	Karsavuran et al. (2014)
	0.8[a]	–	–	HPLC-DAD	Dry matter	–	Bolarinwa et al. (2014)
	–	1.2	2.8	Acid hydrolysis method	With skin	0.6	FSANZ (2014)
	–	0.05	0.4		Without skin		
	3.2[c]	–	–	Acid hydrolysis, subsequent distillation of liberated HCN and titration (iodide/silver nitrate) (VDL-UFA, 1976)	–	0.4	Abraham et al. (2016)
Reported as sweet	0[a]	0[a]	0[a]	Acidic titration method (AOAC, 1980)[b]	Dry matter, without skin	0.6	Femenia et al. (1995)
	0.5[a]	0.2[a]	1.7[a]	HPLC-DAD	Dry matter	–[d]	Yildirim and Askin (2010)
	0.01[a]	< 0.001[a]	0.02[a]	HPLC-DAD	Dry matter	–[d]	Karsavuran et al. (2014)

HCN: hydrocyanic acid; HPLC-DAD: high performance liquid chromatography with diode-array detection.
(a): Estimated from the concentration of amygdalin according to the JECFA conversion factor: 1 g of amygdalin yields 59 mg HCN; the CONTAM Panel is aware of the fact that the molecular weights of CN^- and HCN are different and that the cyanide in the apricot kernels is a mixture of both HCN and CN^-. Thus, depending on the ratio of HCN and CN^-, the actual cyanide concentrations might vary slightly.
(b): Limit of detection and limit of quantification not specified in the original article.
(c): Kernels selected for cyanide content within a specified range, and therefore not necessarily representative.
(d): Data not provided in the original article.

The CONTAM Panel notes that there is substantial variation in cyanide content within kernels described both as 'sweet' and as 'bitter'. However, there appears to be no objective criteria to discriminate between sweet and bitter kernels and the cyanide content in these groups overlaps.

3.4. Food processing

The Mintel Global New Products Database (GNPD),[19] a worldwide market research firm database, and websites were consulted in order to obtain information on the availability of apricot kernels and products thereof on the European market. Several raw apricot kernel products intended for human consumption have been identified. These include whole apricot kernels (with and without skin), apricot kernel butter, apricot kernel powder, ground apricot kernel capsules, apricot kernel nut bars, apricot kernel oil capsules and cold-pressed apricot kernel oil.

Only a few relevant investigations studying the amygdalin/cyanide content of processed raw apricot kernels have been identified by the CONTAM Panel.

In general, processing of CNG-containing plant material such as crushing, grinding, grating, soaking, fermenting and drying reduces cyanide toxicity either through loss of water-soluble glycosides or production of cyanide by action of plant or microbial enzymes and consequent loss through evaporation prior to consumption (Bolarinwa et al., 2014).

Tuncel et al. (1990) investigated the effects of grinding, soaking and cooking on the degradation of amygdalin present in bitter apricot kernels. Their results indicate that endogenous β-glycosidase activity results in a substantial degradation of amygdalin during grinding and subsequent soaking. Glycosides degraded faster in finer particles as compared to coarse ones. They reported that cooking (at 35°C) leads to a substantial reduction of non-glycosidic cyanogens already after 5 min while complete removal of the glycosidic fraction was not achieved even after 30 min.

Peeling also influences cyanide concentration in apricot kernels. A study of cyanide levels in apricot kernels with and without skin found that the levels in apricot kernels with skin ranged from 1,240 to 2,820 mg/kg, whereas in those without skin the levels ranged from 49 to 440 mg/kg (FSANZ, 2014). Higher levels of cyanide detected in apricot kernels with skin may indicate that amygdalin is concentrated within the skin. The processing methods used to remove the skin may also contribute to a reduction in cyanide concentrations (FSANZ, 2014).

However, taking into account that a cyanide concentration of up to 3,800 mg/kg has been reported in apricot kernels without skin (Femenia et al., 1995), the CONTAM Panel concluded that the data available were insufficient to distinguish between the cyanide content of kernels with and without skin.

Grinding of kernels is likely to result in lower amygdalin concentrations due to its hydrolysis by endogenous plant enzymes. The concentration of cyanide is expected to temporarily increase and will subsequently decrease due to the volatility of HCN. However, this has not been quantified.

3.5. Exposure assessment

3.5.1. Previous acute exposure assessments

Apricot kernels are consumed by a very limited part of the population. Due to this, consumption of apricot kernels is not recorded in food consumption databases, and if present, the number of persons is likely to be too limited, and thus not representative, for use in an exposure assessment. Also, the availability of data on cyanide levels in apricot kernels is limited. In previous exposure assessments performed by COT (2006) and FSANZ (2014), exposure assessments were therefore based on numbers of kernels promoted to be consumed to obtain a 'beneficial' health effect combined with fixed concentrations of cyanide in apricot kernels.

The COT considered a consumption of 5 kernels per hour with a maximum of 10 per day based on information from a retailer. Based on information obtained from the Internet, consumption of 30 and 50 kernels per day was also considered. Combined with a mean cyanide concentration of 0.5 mg per kernel (no information on individual kernels was available to COT), the acute exposure to cyanide was estimated at 42 μg/kg bw per hour with a maximum of 83 μg/kg bw per day, and 250 and 417 μg/kg bw per day, respectively (COT, 2006).

FSANZ (2014) estimated the exposure to cyanide via the consumption of four (previously considered by FSANZ to be safe) and 32 kernels per day (promoted maximum intake provided on the Internet) combined with a cyanide concentration of either 2,820 mg/kg in apricot kernels with skin or 440 mg/kg in apricot kernels without skin. These concentrations were the upper levels of a range of concentrations analysed by FSANZ in 18 samples of apricot kernels with skin and 11 samples without skin. One kernel was assumed to weigh 0.6 g. The resulting acute exposure estimates related to the

[19] Mintel Global New Products Database (http://www.mintel.com/global-new-products-database). Accessed: 29 September 2015.

consumption of 4 kernels per day equalled 91 (based on average body weight of 74 kg in persons aged 17 and above in Australia) or 94 μg/kg bw per day (based on average body weight of 71 kg in persons aged 15 and above in New Zealand) for apricot kernels with skin and 14 or 15 μg/kg bw per day for those without skin, respectively. Corresponding acute exposure estimates for 32 kernels per day were 724 or 755 and 113 or 118 μg/kg bw per day, respectively.

3.5.2. Current exposure assessment

The dietary exposure to cyanide via the consumption of raw apricot kernels was estimated separately for toddlers and adults using the highest promoted portion size of 10 kernels per day for the general population and of 60 apricot kernels per day for cancer patients (see Section 2.2.3).

Because very few data were available, two different scenarios were calculated based on the lower and upper mean cyanide concentration levels in bitter apricot kernels reported in the literature, in order to give an indication of the possible range of exposure (see Section 2.2.3). Mean values were considered appropriate for both scenarios, as the cyanide content of individual kernels varies and if a large number of kernels are consumed, not all will contain cyanide at the maximum reported level. The data for apricot kernels described as sweet were too uncertain for use in exposure calculations because the concentrations ranged from undetected up to within the range reported for apricot kernels described as bitter.

In the first scenario, the concentration of cyanide was equal to 0.8 mg/g kernel while in the second scenario the concentration of cyanide was equal to 3.3 mg/g kernel (Table 3).

Kernels were assumed to have an average weight of 0.5 g in both scenarios, based on the midpoint of the range of reported mean kernel weight as presented in Table 2.

The results from the two exposure scenarios, in toddlers and in adults, separately for the general population and cancer patients, are reported in Table 3.

Table 3: Estimated acute exposure to cyanide (μg/kg bw) for toddlers (12 kg bw) and adults (70 kg bw) via the consumption of raw apricot kernels, assuming that apricot kernel weight was equal to 0.5 g

Age group	Acute exposure to cyanide (μg/kg bw)			
	General population (10 kernels per day)[a]		Cancer patients (60 kernels per day)[b]	
	First exposure scenario (μg/kg bw)	Second exposure scenario (μg/kg bw)	First exposure scenario (μg/kg bw)	Second exposure scenario (μg/kg bw)
Toddlers (1–3 years)	333	1,375	2,000	8,250
Adults (18–64 years)	57	236	343	1,414

bw: body weight.
(a): Highest daily portion size of raw apricot kernels promoted for general (healthy) population.
(b): Highest daily portion size of raw apricot kernels promoted for cancer patients.

3.6. Risk characterisation

3.6.1. Risk characterisation based on the consumption of 10 and 60 kernels per day

Due to the limited available data on concentrations of cyanide in apricot kernels and consumption thereof, the CONTAM Panel considered the possible cyanide exposure that might arise from consumption of the numbers of kernels promoted as healthy for the general population and for cancer patients, together with the reported range of cyanide content of bitter apricot kernels, which also encompasses the cyanide content of some apricots described as sweet. These estimated ranges exceed the ARfD of 20 μg/kg bw for both the general population and cancer patients in toddlers and in adults.

For toddlers, consumption of 10 kernels per day could lead to an exposure 17–69 times higher than the ARfD, and consumption of 60 kernels per day could lead to an exposure 100–413 times higher than the ARfD. For adults, consumption of 10 apricot kernels per day could result in an exposure from 3 to 12 times higher than the ARfD, while if 60 kernels per day are consumed, the exposure could be 17–71 times higher than the ARfD. Such exceedances are likely to result in cyanide toxicity in some individuals, as is evident from the case reports of human poisoning. The potential exceedance by other age groups would be between those for toddlers and adults.

3.6.2. Estimation of an amount of apricot kernels that could be consumed without exceeding the ARfD

In order to provide information that might be useful for risk managers, the Panel performed an 'exposure back-calculation' estimating the maximum quantity of apricot kernels (expressed as both grams and number of kernels/day) that can be ingested without exceeding the ARfD of 20 μg/kg bw for toddlers and adults. This calculation was performed using the highest concentration value of cyanide in apricot kernels reported in the literature which is equal to 3.8 mg/g (see Section 3.3), a value that is relevant to the consumption of a single kernel. Extrapolation to number of kernels is based upon the range of individual kernel weights (0.12–0.84 g) reported by Abraham et al. (2016).

As shown in Table 4, the estimated maximum amount of apricot kernels that can be consumed by a toddler is 0.06 g, while an adult could consume up to 0.37 g of kernel per day without exceeding the ARfD, provided the cyanide content in the kernels does not exceed 3.8 mg/g. It is not possible for consumers to measure such small amounts in the home. For a toddler, the ARfD would be exceeded by the consumption of less than one kernel. For adults, three very small kernels could be consumed without exceeding the ARfD, but consumption of less than one half of one large kernel could also exceed the ARfD.

Table 4: Estimated maximum amount and number of raw apricot kernels (g/day) that can be consumed by toddlers and adults without exceeding the ARfD of 20 μg/kg bw

Age group	Body weight (kg)	Cyanide concentration (mg/g)	Maximum amount of apricot kernels (g kernels/day) that can be consumed without exceeding the ARfD	Number of apricot kernels that can be consumed without exceeding the ARfD[(a)]
Toddlers (1–3 years)	12	3.8	0.06	0.1–0.5
Adults (18–64 years)	70		0.37	0.4–3.1

ARfD: acute reference dose.
(a): Assuming a kernel weight of 0.84 g is at the lower end of the range and of 0.12 g at the upper end of the range.

The CONTAM Panel noted that due to the lack of cyanide occurrence data in products derived from raw apricot kernels, the maximum amount of raw apricot kernels that can be consumed without exceeding the ARfD expressed as g kernels/day should be also applied to products derived from raw apricot kernels. This includes ground apricot kernels, which could potentially release the maximum amount of cyanide when freshly ground although over time the cyanide content could decrease due to volatilisation, but there are no data to make a distinction between whole and ground kernels. Moreover, no distinction could be made between bitter and sweet apricot kernels, or between apricot kernels with and without skin due to the lack of adequate data.

3.7. Uncertainties

The evaluation of the inherent uncertainties in the assessment of the acute health risks related to the presence of CNGs in raw apricot kernels and products derived from raw apricot kernels was performed following the guidance of the opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report 'Characterizing and Communicating Uncertainty in Exposure Assessment' was considered (WHO/IPCS, 2008).

3.7.1. Assessment objectives

The objectives of the assessment are clarified in Section 1.2.

3.7.2. Occurrence data/exposure assessment

No occurrence data on amygdalin or cyanide (originating from amygdalin) in raw apricot kernels were available in the EFSA occurrence database. From the published literature, only very limited occurrence data on amygdalin or cyanide in apricot kernels or products were available, which were also very variable (Table 2). To address this uncertainty, two exposure scenarios were defined to

assess the exposure to cyanide using either a lower or upper mean concentration as reported in the literature (Section 3.3). For the estimation of the maximum weight of kernels that can be consumed without exceeding the ARfD (Section 3.6.2), the highest reported concentration of cyanide in apricot kernels was used (Table 2). Due to the limited data available, it is not unlikely that actual cyanide levels in apricot kernels may be higher than the value used in this opinion. Due to this, the maximum weight of kernels that can be consumed safely may in practice be lower than the weight estimated here, which may have resulted in an underestimation of the risk.

In the EFSA database, also no consumption data on raw apricot kernels and products derived thereof were available. Therefore, the CONTAM Panel used the consumption amounts cited on the Internet to carry out exposure assessments for the general population and cancer patients. The extent to which consumers follow the Internet recommendations is unknown.

There was also only very limited information available about the effects of processing on amygdalin/cyanide content of apricot kernels. Therefore, the exposure assessment was based on data for whole kernels, which is likely to overestimate exposure from products prepared from apricot kernels.

The publicly available information did not provide objective criteria to discriminate between amygdalin/cyanide concentrations in sweet and bitter apricot kernels. In addition, amygdalin/cyanide contents in kernels reported as 'bitter' or 'sweet' overlapped. The lowest and highest mean values reported in kernels described as bitter were used for the assessment in order to give an indication of the possible exposure when consuming a large quantity of kernel. There is considerable uncertainty about possible concentrations outside this range.

The weight of individual apricot kernels varies considerably. For the exposure assessment, the midpoint of the published information on kernel weights was used, which might not reflect the actual weights of kernels consumed. In reality, the actual kernel weight can be either lower or higher. The estimated exposures to cyanide based on number of kernels consumed (10 and 60) could therefore have been overestimated or underestimated, respectively.

In estimating the maximum number/weight of kernels that could be assumed without exceeding the ARfD, the range of individual kernel weights in one batch of kernels was used in order to reflect the uncertainty. However, it is possible that other kernels would be outside this weight range. Furthermore, the maximum reported occurrence value of amygdalin cyanide was used. Because of the very limited amount of data available, this maximum reported value could be lower than actually occurs on the market. Therefore, the maximum weight of kernels that can be consumed without exceeding the ARfD could be overestimated but not underestimated.

3.7.3. Other uncertainties

The assumption that a blood cyanide concentration of 20 μM is the threshold for toxicity in humans was used as a basis for deriving the ARfD, although the original data underlying this assumption were not available. This value of 20 μM would be an underestimate if, as is likely, the measurements were made some time after peak blood cyanide concentrations would have occurred. However, there is no information on whether a back-calculation was made to allow for the rapid elimination of cyanide, nor of the number of individuals that the value is based on, and whether it covers potentially sensitive subgroups.

The mean data from five females in a study indicating 100% bioavailability were used to extrapolate the blood cyanide concentration of 20 μM to an external dose of total cyanide. Because the effects of cyanide are due to peak blood concentrations, which occur rapidly after ingestion of bitter apricot kernels, interindividual differences due to toxicokinetic variability are likely to be smaller than the default value of 3.16 applied for differences in absorption, distribution, metabolism and elimination. The variations in peak blood levels seen in the study from Abraham et al. (2016) were small. Women have a smaller distribution volume of blood than men and children have a larger blood volume (per kg/bw) as than adults. Therefore, a factor of 1.5 was considered sufficient to cover any additional variability in toxicokinetics. This could be an underestimation or overestimation of actual variability.

The uncertainty with respect to extrapolation to more sensitive individuals was addressed by applying the default UF of 3.16 for individual variation in toxicodynamics. The Panel noted that this factor might not be fully required as the mode of action of cyanide, involving disruption of cellular energy metabolism, does not indicate major individual variation in sensitivity.

3.7.4. Summary of uncertainties

In Table 5, a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the source of uncertainty leads to overestimation/ underestimation of the resulting risk.

Table 5: Summary of uncertainties in the risk assessment of CNGs in raw apricot kernels and products thereof

Sources of uncertainty	Direction[(a)]
No occurrence data in the EFSA database and consequent need to rely on limited occurrence data published in the literature	+/−
Assumption of complete conversion of amygdalin to cyanide	+
Limited data on the impact of food processing	+
No objective criteria for discriminating between 'sweet' and 'bitter' apricot kernels	+
Lack of consumption data	+/−
Appropriateness of the midpoint of the reported mean kernel weights used in the exposure assessment	+/−
Appropriateness of the range of the mean cyanide concentrations in the exposure assessment	+/−
Assumption that 20 μM cyanide in blood is a threshold for toxicity in humans, including sensitive subgroups	+/−
Selection of an uncertainty subfactor of 1.5 for toxicokinetic variability	+/−
Application of the default uncertainty subfactor of 3.16 for toxicodynamic variability	+
Estimation of the maximum weight of kernels that can be consumed without exceeding the ARfD using the maximum reported occurrence value which might be lower than actual maximum values	−

ARfD: acute reference dose.
(a): +: uncertainty with potential to cause overestimation of exposure/risk; −: uncertainty with potential to cause underestimation of exposure/risk. Extent of potential overestimation/underestimation might differ in direction.

The overall uncertainty incurred with the present assessment is considered as high. The assessment is more likely to overestimate than to underestimate the risk.

4. Conclusions

4.1. General

- Amygdalin is the major cyanogenic glycoside (CNG) present in apricot kernels.
- In intact apricot kernel cells, amygdalin and catabolic enzymes are stored in separate compartments but can be brought into contact by physical processes such as grinding or by chewing, thereby releasing cyanide.
- Complete degradation of 1 g of amygdalin releases 59 mg of hydrocyanic acid (hydrogen cyanide, HCN). HCN is a weak acid and always exists as a mixture of non-dissociated acid and its dissociated form (cyanide ions, CN^-) in aqueous environment. This mixture is referred to in this opinion as 'cyanide'.
- No validated methods are available for the measurement of amygdalin as well as cyanide (originating from amygdalin) in raw apricot kernels or products derived thereof.

4.2. Hazard assessment

4.2.1. Toxicokinetics

- The absorption of intact amygdalin is minimal.
- Cyanide is released from amygdalin in apricot kernels during chewing and in the intestinal tract by the gut microbiota.
- Cyanide is readily absorbed from the intestine, reaching maximum blood levels usually within minutes.
- Cyanide in blood is mostly found in erythrocytes bound to methaemoglobin.

- Cyanide has an initial half-life of about 1 h in blood and is rapidly distributed to all organs. The volume of distribution has been estimated as 0.075 L/kg in humans.
- Thiocyanate is the major metabolite of cyanide, formed primarily in the liver and excreted in the urine.
- The formation of thiocyanate represents a detoxification of cyanide and depends on the activity of certain enzymes and the availability of sulfur-containing amino acids.
- There are no pronounced species differences in cyanide toxicokinetics.
- Cyanide in blood has been frequently used as an exposure biomarker for cyanide poisoning. All steps of storage, sample preparation and the analytic process itself have to be carried out with caution to minimise the risk of cyanide loss and falsely low levels.

4.2.2. Acute and short-term toxicity in animal studies

- The overt clinical effects in experimental animals due to lethal levels of cyanide exposure are: dyspnoea, irregular shallow and gasping breathing, ataxia, tremors, retrocolic spasms, tonic spasms, loss of consciousness, convulsions and asphyxiation. Brain seems to be the organ most sensitive to acute cyanide toxicity.
- Acute medium lethal oral doses (LD_{50}) values for cyanides range from 2.13 to 6 mg/kg body weight (bw).
- Upon short-term dietary exposure to cyanide, histopathological changes and organ weight (e.g. decreased weights of cauda epididymis and thymus, and increased weights of liver) are the parameters most frequently affected in adult rodents.
- A no observed adverse effect level (NOAEL) of 0.36 mg CN^-/kg bw and a lowest observed acute effect level (LOAEL) of 1.2 mg CN^-/kg bw were established from a study in rats exposed to KCN for 15 days, supported by the data from a study in rats dosed with NaCN for 13 weeks.
- Experimental data on amygdalin acute and short-term toxicity are too few to derive any NOAEL and/or LOAEL.
- Indications of teratogenicity in offspring of hamsters treated with amygdalin on day 8 of gestation were observed in one study while signs of toxicity were revealed both in rat dams and their weanling offspring after exposure to KCN. In both studies, the cyanide doses were higher than the NOAEL/LOAEL cited above.

4.2.3. Human observations

- The human acute lethal oral dose of HCN is reported to be 0.5–3.5 mg/kg bw. It is frequently cited that the toxicity threshold for cyanide in whole blood is 0.5 mg/L (approximately 20 micro mol, μM).
- Cases of acute cyanide toxicity have resulted from ingestion of amygdalin preparations and of bitter apricot kernels, and some of these cases were fatal. For adults, the number of kernels consumed was 20 or more. For children, five or more kernels appear to be toxic.
- In a study in a small number of healthy adults, there were no symptoms of cyanide toxicity at cyanide equivalent doses of up to 0.113 mg/kg bw in women and 0.34 mg/kg bw in men (the highest doses tested).

4.2.4. Mode of action for acute toxicity

- The primary mode of action underlying the acute toxicity of cyanide involves inhibition of oxidative phosphorylation, in particular, by inhibiting cytochrome oxidase a3.
- Heart and brain are particularly prone to cyanide toxicity because they require continuous supply of adenosine triphosphate generated by oxidative phosphorylation.
- Cyanide also inhibits several other enzymes and may act via a number of additional mechanisms that contribute to its acute toxicity.

4.2.5. Acute reference dose

- The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that an acute reference dose (ARfD) should be established for cyanide in order to consider the acute health risks related to CNGs in apricot kernels.
- The animal data did not provide a suitable basis for deriving an ARfD.

- The CONTAM Panel concluded that the blood cyanide concentration of 20 μM, which is reported to be a threshold for toxicity, should be used as the basis for deriving the ARfD, extrapolating to an external dose of cyanide.
- In a bioavailability study, a group of 12 healthy adult volunteers consumed CNGs corresponding to 6.8 mg cyanide from a number of different sources including bitter apricot kernels. A cyanide exposure of 0.105 mg/kg bw was estimated to result in a mean peak blood level of 20 μM in the females in the study.
- The CONTAM Panel concluded that the dose of 0.105 mg/kg bw resulting in a blood concentration of about 20 μM in the females should be used as the basis for deriving the ARfD.
- Assuming that CNGs are completely broken down to cyanide, and that the cyanide is rapidly absorbed and 100% bioavailable, it is highly unlikely that individual variability could lead to appreciably higher peak blood levels. Because of the very short time to peak blood level, individual differences in metabolism (e.g. due to reduced activity of rhodanese) could delay elimination, but are unlikely to have an impact on the peak level. The variations in peak blood levels seen in the study from Abraham et al. (2016) were small. Women have a smaller distribution volume of blood than men and children have a larger blood volume (per kg/bw) than adults. Therefore, the CONTAM Panel concluded that a default factor of 3.16 was not required and that a factor of 1.5 was sufficient to cover any additional variability in toxicokinetics.
- In considering a need for a toxicodynamics subfactor, the CONTAM Panel noted the lack of information on whether potentially sensitive individuals (e.g. children) were included in the database underpinning the assumption that a blood cyanide level of 20 μM is a toxicity threshold, and that the bioavailability study was conducted in a small number of healthy volunteers. The CONTAM Panel concluded that a toxicodynamic subfactor should be applied, and in the absence of cyanide-specific data on individual sensitivity, adopted the default subfactor of 3.16.
- Dividing the NOAEL of 0.105 mg/kg bw by the factors of 1.5 and 3.16, with rounding to a single significant figure, an ARfD for cyanide of 0.02 mg/kg bw (20 μg/kg bw) is established for use in assessing the risks associated with the presence of CNGs in apricot kernels.

4.3. Exposure assessment

- There is substantial variation in cyanide content within kernels described both as 'sweet' and 'bitter'. However, there appears to be no objective criteria to discriminate between sweet and bitter kernels and the cyanide content in these groups overlaps.
- In the absence of consumption data, the highest number of kernels promoted for the general population and cancer patients (10 and 60 kernels, respectively) by apricot kernel vendors were used for exposure assessment together with the highest and lowest mean values for cyanide concentrations in apricot kernels described as 'bitter'.
- For the general population, resulting exposures were 333 and 57 μg/kg bw assuming low cyanide content and 1,375 and 236 μg/kg bw assuming high cyanide content, for toddlers and adults, respectively. For cancer patients, the corresponding numbers were 2,000 and 343 μg/kg bw and 8,250 and 1,414 μg/kg bw.
- Given the lack of consumption data and the very limited information on cyanide in apricot kernels, the exposure estimates of cyanide reported in this opinion are highly uncertain. The real exposure in a specific consumer may be either higher or lower, but, given the uncertainties, it is estimated that the real exposure is more likely to be lower than higher than the intakes reported here.

4.4. Risk characterisation

- If consumers follow the recommendations of websites that promote consumption of apricot kernels, their exposure to cyanide will greatly exceed the ARfD. Such exceedances are likely to result in cyanide toxicity in some individuals, as is evident from the case reports of human poisoning.
- The estimated maximum quantity of apricot kernels that could be eaten by a toddler is 0.06 g, while an adult could eat up to 0.37 g of kernel per day without exceeding the ARfD. It is not possible for consumers to measure such small quantities in the home.

- For a toddler, the ARfD would be exceeded by the consumption of less than one kernel. For adults, very small kernels could be consumed without exceeding the ARfD, but consumption of one large kernel could also exceed the ARfD.
- The CONTAM Panel was not able to separately characterise risks associated with sweet raw apricot kernels, products containing raw apricot kernels or processed raw apricot kernels (e.g. ground, without skin, etc.) due to the lack of adequate data.

5. Recommendations

- Information is needed on whether there is an objective distinction between 'sweet' and 'bitter' apricot kernels, and if so whether the cyanide contents differ.
- Data are needed on the occurrence of cyanide in whole raw apricot kernels and products derived from them.
- More information is required on the impact of removing the skin and other forms of processing on the cyanide content.
- Data are required on consumption of products prepared from raw apricot kernels.

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Abbreviations

α-KG	α-ketoglutarate
α-KGCN	α-ketoglutarate cyanohydrin
ADP	adenosine diphosphate
AFC Panel	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
AH	amygdalin hydrolase
ALT	alanine aminotransferase
ARfD	acute reference dose
ATCA	2-amino-2-thiazoline-4-carboxylic acid
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
BfR	German Federal Institute for Risk Assessment
bw	body weight
BMD	Benchmark dose
$BMDL_{10}$	95% lower confidence limit for the benchmark dose response of 10% extra risk
CAS	Chemical Abstracts Service
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
CNG	cyanogenic glycoside
COT	UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CYP	cytochrome P450
DECOS	Dutch Expert Committee on Occupational Standards
EC	European Commission, Enzyme commission number
EFET	The Hellenic Food Authority
ELISA	enzyme-linked immunosorbent assay
FAO/WHO	Food and Agriculture Organization of the United Nations/World Health Organization
FSANZ	Food Standards Australia New Zealand
GC	gas chromatography
GC-MS	gas chromatography mass spectrometry
GD	gestation day
GC-ECD	gas chromatography-electronic capture detection
GC-MPD	gas chromatography-microwave-induced plasma atomic emission detection
HCN	hydrocyanic acid
HPLC	high performance liquid chromatography
HPLC-DAD	high performance liquid chromatography with diode-array detection

HPLC-MS/MS	high performance liquid chromatography-tandem mass spectrometry
HPLC-UV	high performance liquid chromatography with UV detection
i.v.	intravenous
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KCN	potassium cyanide
LC	liquid chromatography
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LD_{50}	median lethal dose
LOAEL	lowest observed adverse effect level
LOD	limit of detection
MNL	mandelonitrile lyase
MPST	mercaptopyruvate sulfurtransferase
NADH	nicotinamide adenine dinucleotide
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
PH	prunasin hydrolase
PMTDI	Provisional Maximum Tolerable Daily Intake
PND	postnatal day
SD	standard deviation
TSH	thyroid stimulating hormone
UF	uncertainty factor
UGT	uridine diphosphate glucose-glucosyl transferase
US EPA	US Environmental Protection Agency
UV	ultraviolet
VDL-UFA	Association of German Agricultural Analytic and Research Institutes
WHO	World Health Organization

Appendix A – Identification and selection of relevant scientific literature and reports

Table A.1: Search terms and information source for literature search on cyanide

Chemistry and analysis		
Search terms	TOPIC: ('hydrocyanic acid' OR 'cyanogenic glycosides' OR amygdalin OR prunasin OR prussic acid OR laetrile OR formonitrile OR 'Vitamin B17')	TOPIC: (bitter apricot OR apricot paste)
	AND TOPIC: (chemistry OR analysis OR determination OR detection OR identification OR formation OR GC OR GC-MS OR HPLC OR LC-MS OR ICP-MS)	
Information source	**Number of records retrieved**	
Web of Science	885	30
PubMed	221	30
Formation, Occurrence, Exposure		
Search terms	TOPIC: ('hydrocyanic acid' OR 'cyanogenic glycosides' OR amygdalin OR prunasin OR prussic acid OR laetrile OR formonitrile OR 'Vitamin B17')	TOPIC: (bitter apricot OR apricot paste)
	AND TOPIC: (occurrence OR exposure OR assessment OR levels OR concentrate* OR raw apricot kernels)	
Information source	**Number of records retrieved**	
Web of Science	628	143
PubMed	80	7
Metabolism, Kinetics		
Search terms	TOPIC: ('hydrocyanic acid' OR 'cyanogenic glycosides' OR amygdalin OR prunasin OR prussic acid OR laetrile OR formonitrile OR 'Vitamin B17')	TOPIC: (bitter apricot OR apricot paste)
	AND TOPIC: (toxicokinetic* OR metabolism OR distribution OR excretion OR absorption OR distribution OR biomarker OR mode of action OR biotransformation OR elimination OR reduction OR detoxification OR extraction)	
Information source	**Number of records retrieved**	
Web of Science	605	166
PubMed	221	25
Food processing		
Search terms	TOPIC: ('hydrocyanic acid' OR 'cyanogenic glycosides' OR amygdalin OR prunasin OR prussic acid OR laetrile OR formonitrile OR 'Vitamin B17')	TOPIC: (bitter apricot OR apricot paste)
	AND TOPIC: (ground OR powder OR milled OR flour OR cracked OR chopped OR hulled OR unhulled OR 'with skin' OR 'without skin')	
Information source	**Number of records retrieved**	
Web of Science	175	53
PubMed	18	4

Toxicity		
Search terms	TOPIC: ('hydrocyanic acid' OR 'cyanogenic glycosides' OR amygdalin OR prunasin OR prussic acid OR laetrile OR formonitrile OR 'Vitamin B17')	TOPIC: (bitter apricot OR apricot paste)
	AND TOPIC: (toxicity OR toxi* OR acute OR subacute OR subchronic OR chronic OR mutagen* OR carcino* OR genotox* OR reprotox* OR nephrotox* OR neurotox* OR hepatotox* OR immunotox* OR haemotox* OR hematotox* OR cytotox* OR develop* toxicity OR thyroid OR endocri* OR poisoning OR incidental poisoning OR rat OR mouse OR lab animal OR animal* OR case studies)	
Information source	**Number of records retrieved**	
Web of Science	899	205
PubMed	135	15
Epidemiology		
Search terms	TOPIC: ('hydrocyanic acid' OR 'cyanogenic glycosides' OR amygdalin OR prunasin OR prussic acid OR laetrile OR formonitrile OR 'Vitamin B17')	TOPIC: (bitter apricot OR apricot paste)
	AND TOPIC: (biomarker OR biological marker OR case studies OR incidental poisoning OR poisoning OR human poisoning)	
Information source	**Number of records retrieved**	
Web of Science	209	48
PubMed	41	5
Date accessed	**4 June 2015**	**4 September 2015**
Total number retrieved	**4,117**	**731**
Number after duplicate removal	**1,733**	**401**

Appendix B – EFSA guidance documents used for the assessment

- EFSA (European Food Safety Authority), 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. doi:10.2903/j.efsa.2010.1557
- EFSA (European Food Safety Authority), 2011. Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances. EFSA Journal 2011;9(12):2490, 33 pp. doi:10.2903/j.efsa.2011.2490
- EFSA (European Food Safety Authority), 2011. Use of BMDS and PROAST software packages by EFSA Scientific Panels and Units for applying the Benchmark Dose (BMD) approach risk assessment. Technical Report. EN-113. 190 pp.
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