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CYANIDE POISONING: FROM PHYSIOLOGY TO FORENSIC ANALYTICAL CHEMISTRY

Andriana SURLEVA[1*], Robert GRADINARU[2], Gabi DROCHIOIU[2*],

[1)] Department of Analytical Chemistry, University of Chemical Technology and Metallurgy, 8 "Kl. Ohridski" blvd., 1756 Sofia, Bulgaria
[2)] Faculty of Chemistry, "Al. I. Cuza" University, 11 Carol I, Ro-700506 Iasi, Romania

Abstract
The extreme toxicity of cyanide, its wide industrial application as well as its continued illegal use generate research interest in different fields of science, imposing multidisciplinary approach to study cyanide poisoning. This review presents new data about cyanide exposure, toxicology, and antidote development. Cyanide concerned research in environmental and forensic sciences along with medicine closely depends on the recent achievements in cyanide determination methods. Newly reported cyanide detection systems and sample pretreatment procedures for environmental, biological and plant samples are summarized. The main requirements to analytical systems for cyanide determination and the trends in analytical research are also discussed.

Keywords*: cyanide poisoning; cyanide determination; cyanide antidotes.*

Introduction

Cyanides comprise a wide range of compounds of varying degrees of chemical complexity and toxicity, all of which contain a CN moiety, to which humans are exposed in gas, liquid, and solid form from a broad range of natural and anthropogenic sources. Daily, people may be exposed to low levels of cyanides from foods, smoking and other sources. Lethal exposures to cyanides result only from accidents, suicides or homicides. Inhalation of cyanide gas, especially within an enclosed space, poses a significant health risk. Ingestion of food and beverages containing cyanide can also cause serious health effects.

Cyanide has been used as a poison for thousands of years. Since the time of ancient Egypt, plants containing cyanide derivatives, such as bitter almonds, cherry laurel leaves, peach pits, and cassava, have been used as lethal poisons (Sykes, 1981). Peach pits used in judicial executions by the ancient Egyptians are on display in the Louvre Museum, Paris, and an Egyptian papyrus refers to the "penalty of the peach." The Romans used cherry laurel leaves as a method of execution (also known as "the cherry death"). For the first time cyanide was produced expressly for the purpose of killing during World War I, in late 1915 and early 1916 (Baskin et al., 2008).

During World War II, the Nazis were considered to employ HCN (Zyklon B) to exterminate people in the concentration camps. Cyanide was detected in the walls of crematoria almost 50 years later (Baskin, 2001). Cyanide has

been the typical agent used in "gas chambers" for judicially execution of murderers, in which a cyanide salt is dropped into an acid to produce HCN. Cyanide has often been used by individuals and groups to commit suicide. One of the most notorious of such events happened in 1978 near Port Kaituma, Guyana, when the followers of Jim Jones drank a grape-flavored drink laced with cyanide, resulting in the deaths of more than 900 children and adults (Thompson *et al.*, 1987).

Nowadays, sodium cyanide has been still used illegally for fishing in some south-east Asia countries. Cyanide fishing is a fast method to stun and collect fish, but practice causes irreversibly damaging of the coral reefs. Since the 1960s, it is estimated that over 1 million kilograms of cyanide have been used on the Philippine reefs (Mak *et al.*, 2005a).

The annual production of KCN is about 1.4 million tons and 13% of it is used in refining metallurgical processes. Although the cyanide-containing water discharge is strictly regulated and pre-treatment procedures are strongly recommended, some industrial accidents and illegal wastes discharge have been reported (www.rainforestinfo.org.au).

Cyanide poisoning presents one of the most difficult challenges in disaster medicine and forensic science, due to its high toxicity, fast acting, a number of possible sources of exposure and some limitations of analytical methods for cyanide determination. The aim of this review is to summarize the main anthropogenic and natural sources of cyanide releasing into environment, biochemical basis of cyanide poisoning and available antidotes for its treatment. The recent achievements in cyanide determination in biological fluids, environmental objects and plants are also reviewed and trends in method development are discussed.

1. Cyanide exposure

Cyanide containing compounds, mainly hydrogen cyanide and sodium or potassium cyanides, are widely used in the industry: in ore extracting processes for the recovery of gold and silver, electroplating, case-hardening of steel, base metal flotation, metal degreasing, dyeing, and printing, in the production of chelating agents, in the synthesis of organic and inorganic chemicals. Hydrogen cyanide is also used as a fumigant in ships, railroad cars, large buildings, grain silos, and flour mills, as well as in the fumigation of peas and seeds in vacuum chambers.

Anthropogenic sources of cyanide release to the environment are diverse: gaseous waste or waste water from manufacturing and processing industries, emissions from municipal solid waste incinerators, biomass burning, fossil fuel combustion, including vehicle emissions (Baum *et al.*, 2007), fumigation operations, and the production of coke or other coal carbonization procedures. Hydrogen cyanide is formed during the incomplete combustion of nitrogen-containing polymers, such as certain plastics, polyurethanes, and wool (Koskinen-Soivi *et al.*, 2005).

Hydrogen cyanide is present in cigarette smoke (Xu *et al.*, 2006; Brunnemann *et al.*, 1977). It is one of the 44 harmful substances in cigarette smoke which inhibits several respiratory enzymes and is a major ciliatoxic agent, which cause changes in the epithelial lining of certain organs of the body. The amount of cyanide in cigarette smoke might directly affect people's health, especially the

central nervous system. Studies of workers exposed chronically to hydrogen cyanide have reported a range of nonspecific neurological effects that include headache, dizziness and paresthesiae (Pritchard, 2007). These features may persist after discontinuation of exposure.

Principal natural sources of cyanides are over 2600 plant species, including fruits and vegetables that contain cyanogenic glycosides (cyanogens), which can release cyanide on hydrolysis when ingested (Ganjewala *et al.*, 2010; Bjarnholt *et al.*, 2008; Barceloux, 2008). Among them, cassava (tapioca, manioc) and sorghum are staple foods for millions of people in many tropical countries. Hydrogen cyanide is released into the atmosphere also from natural biogenic processes from higher plants, bacteria, and fungi.

The majority of the population is exposed to very low levels of cyanide in the general environment. There are, however, specific subgroups with higher potential for exposure. These include individuals involved in large-scale processing of cassava and those consuming significant quantities of improperly prepared foods containing cyanogen glycosides (WHO, 2004). The cassava root (tapioca) contains a sufficient amount of cyanogens to require special processing to reduce the danger of toxicity (Bardbury & Denton, 2011). The maximum permissible limit of cyanogen content in cassava flour is 10 mg HCN/kg (WHO, 2004). The edible portions of dietary plant species commonly used in the European countries contain relatively low levels of cyanogen glycosides, although some pits and seeds of common fruits contain significantly higher concentrations.

The cyanogens content of apricot and choke cherry kernels is high enough to cause acute intoxication, especially in children (WHO, 2004; Akyildiz *et al.*, 2010; Barceloux, 2008). Cyanide was also found in canned stone fruits (Barceloux, 2008; WHO, 2004). A dangerous dose of 20 almond kernels containing 29 mg HCN/kg have been recently reported (Morandini, 2010). Livestock poisoning due to cyanogenic glycoside dhurrin in sorghum and in sudangrass is well documented (Goff *et al*., 2011). Flaxseed is a multi-purpose crop and its consumption is beneficial for human health, but some cultivars contain high concentration of cyanogens that restrict their daily dose or their use in animal feed mixtures (Herchi *et al*., 2012; Bacala & Barthet, 2007).

Other subgroups with greatest potential for exposure include those in the vicinity of accidental or intended releases from point sources, active and passive smokers, and fire-related smoke inhalation victims. Workers may be exposed to cyanides during fumigation operations and the production and use of cyanides in many industrial processes (WHO, 2004). Probably the commonest cause of cyanide poisoning in the western world is through inhaled smoke in confined spaces during fires affecting domestic and industrial buildings (Lindsay *et al.*, 2004). The majority of recent studies support blood cyanide concentration of less than 0.026 μg/mL in healthy subjects. Raised blood cyanide concentration is a clinical feature of smoke inhalation and inhaled hydrogen cyanide gas may prove to be fatal. In fire death cases, toxicological data from the victims, such as their carboxyhemoglobin and blood cyanide levels, can provide the Fire Investigator with important scientific evidence to further a determination of the

origin and cause of the fire. As discussed in Guide for Fire and Explosion Investigations (NFPA 921, 2008): a relationship exists between the nature of a fire, i.e., smoldering, flaming, post-flashover, and the production of toxic gases such as carbon monoxide and hydrogen cyanide. However, cyanide stability comes into question when the investigator has to interpret toxicological results from fire victims due to changes in cyanide concentration over time in postmortem victims and stored blood samples (McAllister *et al.*, 2008). Different cyanide exposure models to car emissions in open and closed areas have also been proposed by Baum *et al*. (2007). The cyanide concentration in air above the acute toxicity level was obtained for a residential garage model: 192 μg HCN/m^3 over 3 h of an idle-running car.

Some drugs contain cyanide or substances which can be converted to cyanide within the body, for example, sodium nitroprusside ($Na_2Fe(CN)_5NO$) which is sometimes administered intravenously during the critical care treatment of hypertension. However, toxic effects of this drug have been reported (Sani *et al*., 2011), originally ascribed to the nitroso moiety or to various decomposition products such as cyanide, thiocyanate, and nitrite. It was postulated that the iron atom of the nitroprusside complex reacts with the free sulfhydryl groups (-SH) in erythrocytes and releases cyanide *in vivo* by nonenzymatic reaction.

Cyanide salts such as sodium cyanide (NaCN) and potassium cyanide (KCN) are associated with ingestive poisoning. Cyanides are used as suicidal but also as homicidal agents (Gill *et al*., 2004; Musshoff *et al.*, 2011), particularly among healthcare and laboratory workers, and they can potentially be used in a terrorist attack. It is also still used in cases of illegal euthanasia (Blanco & Rivero, 2004). Recently, a case report on a person who was not very familiar with chemicals, especially not with cyanides, has demonstrated the acquisition of professional information via the internet, enabling a suicide with a complex procedure by inhalation of HCN (Musshoff *et al.*, 2011).

2. Biochemical basis for cyanide poisoning

Cyanides are well absorbed via the gastrointestinal tract or skin and rapidly absorbed via the respiratory tract. Once absorbed, cyanide is rapidly and ubiquitously distributed throughout the body, although the highest levels are typically found in the liver, lungs, blood, and brain. There is no accumulation of cyanide in the blood or tissues following chronic or repeated exposure (Baskin *et al.*, 2008).

High concentrations of cyanide may produce giddiness, headaches, unconsciousness and convulsions with paralysis of the central respiratory center. Clinical features include coma, respiratory arrest, and cardiovascular collapse. Cyanide ion toxicity is mediated primarily by its high affinity for the ferric moiety of cytochrome *c* oxidase in mitochondria, a key component in oxidative respiration. This stable but reversible interaction blocks the last stage in the electron transfer chain, resulting in cellular hypoxia and shift from aerobic to anaerobic cellular respiration, leading to cellular ATP depletion, lactic acidosis as well as cell and tissue death (Pritchard, 2007).

The most important route of cyanide excretion is by formation of thiocyanate, which is subsequently

excreted in the urine. Thiocyanate formation is catalyzed directly by the enzyme rhodanese and indirectly via a spontaneous reaction between cyanide and the persulfide sulfur products of the enzymes 3-mercaptopyruvate sulfurtransferase and thiosulfate reductase. Minor pathways for cyanide detoxification involve reaction with cystine to produce aminothiazoline- and iminothiazolidinecarboxylic acids and combination with hydroxycobalamin (vitamin B_{12a}) to form cyanocobalamin (vitamin B_{12}); these end-products are also excreted in the urine (WHO, 2004; Baskin *et al.*, 2008). Combined, these metabolic routes detoxify 0.017 mg of cyanide per kilogram of body weight per minute in the average human (1.19 mg/min in a 70-kg person) (Baskin *et al.*, 2008; Lindsay *et al.*, 2004).

After a single brief exposure to a low concentration of hydrogen cyanide from which an individual recovers quickly, no long term health effects are anticipated. Intoxication following deliberate ingestion of sodium or potassium cyanide has been reported to cause severe neurological impairment. A slow recovery from severe dystonia syndromes arising from cyanide intoxication has been noted in some cases (Pritchard, 2007).

Cyanide is one of the few chemical agents that does not follow Haber's law, which states that the C*t* (the product of concentration and time) necessary to cause a given biological effect is constant over a range of concentrations and times; for this reason, the LC*t*50 (the vapor or aerosol exposure that is lethal to 50% of the exposed population) for a short exposure to a high concentration is different from a long exposure to a low concentration (Pritchard, 2007).

The biological hallmark for cyanide exposure is lactic acidosis and high concentration of oxyhemoglobin in the venous return. A high plasma lactate concentration in fire victims without severe burns and in pure cyanide poisoned patients is a sensitive and reliable specific indicator of cyanide intoxication (Megarbane *et al.*, 2003). Hydrogen cyanide in breath has been also suggested as a diagnostic tool for cyanide poisoning and for cyanide-producing bacterial infections (Stamyr *et al.*, 2009). The major metabolite of cyanide, thiocyanate, is considered to be more stable than cyanide *in vivo*, but it can be introduced by routes other than cyanide metabolism, making it difficult to use as a marker of cyanide exposure. Cyanide also forms a minor metabolite, 2-amino-2-thiazoline-4-carboxylic acid, which is relatively stable and has good potential as a biomarker for cyanide exposure (Logue *et al.*, 2005; Baskin *et al.*, 2008). Recently, thiocyanate protein adducts has been proposed as a long-term repository for information regarding cyanide exposure (Youso *et al.*, 2010).

3. Cyanide Antidotes

Cyanide produces a rapid onset of toxicity and thus requires vigorous and immediate treatment to prevent the toxic syndrome. Rapid removal from further exposure, administration of general support measures including 100% oxygen, and administration of specific antidotes in critically impaired casualties effectively reverse the effects of exposure. A series of newer antidotes both alone or in conjunction with sodium thiosulfate treatment have been examined and classified into three major groups (Hall *et al*., 2009; Beatriz, 2007; Jones & Scott, 2008):

(i) methemoglobin inducers: sodium nitrite, amyl nitrite, and 4-dimethylaminophenol promote the formation of methemoglobin which binds cyanide and so keeps it from binding to cellular cytochrome oxidase. However, they are reported to be very slow acting and associated with severe side effects (Bhattacharya & Vijayaraghavan, 2002; Pritchard, 2007).

(ii) cobalt containing compounds: dicobalt edetate (cobalt EDTA) and hydroxocobalamin. Cobalt acts as a chelating agent for cyanide, and bounded cyanide is excreted in the urine. Dicobalt edetate have been shown to be potentially toxic, but hydroxocobalamin has recently been approved as a safe and effective cyanide antidote (Des Lauriers *et al*., 2006; Fortin *et al*., 2010);

(iii) cyanohydrin formers: alpha-ketoglutarate reacts with cyanide to form nontoxic cyanohydrin derivatives and its promising role as an alternative treatment for cyanide poisoning has been reported (Bhattacharya & Vijayaraghavan, 2002; Sultana *et al*., 2011; Tulsawani *et al*., 2006).

Many of the existing antidotes for cyanide poisoning are highly toxic themselves particularly when they are given at such doses that there is no remaining cyanide on which they can act (Lindsay *et al.*, 2004). Sometimes the antidotes are given before obtaining the results from blood tests and thus they are in inappropriate quantity. Sometimes the antidote administration is delayed and the damage is done either by cyanide or by antidotes. During the delay between diagnosis and administration, cyanide has been metabolized and the required dose of antidote has invariably altered.

4. Analytical aspects of cyanide poisoning: problems and trends

a) Cyanide determination in environmental samples

The specificity of cyanide as an environmental pollutant is of special concern, due to the different toxicity of cyanide-containing substances, from one side, and from other side, to the fact that the cyanide quantification depends on the analytical method used (Zheng *et al*., 2003). Cyanide pollutants have been officially classified into three main groups depending of their toxicity and environmental fate: (i) free cyanide - including HCN, alkaline and alkaline earth cyanides; (ii) weak acid dissociable cyanide (WAD) - a collective term for free cyanide and metal-cyanide complexes ($Ag(CN)_2^-$, $Cu(CN)_4^{3-}$, $Cd(CN)_4^{2-}$, $Zn(CN)_4^{2-}$, $Hg(CN)_4^{2-}$, $Ni(CN)_4^{2-}$,) which easily release HCN under slightly acidic environmental conditions; and (iii) total cyanide - each potential source of HCN regardless of its origin (U.S. EPA, 1992). The term "cyanide" refers to all CN groups that can be determined analytically as cyanide ion (CN) via spectrophotometric or electrochemical measurements, usually following appropriate sample pre-treatment to release cyanide ion (APHA,1998). The Environmental Protection Agencies have imposed maximum contaminant levels (MLC) for cyanide discharge into the environment. The MLC for WAD cyanide vary from 0.05 to 0.07 μg/L for drinking water and in the range between 200-500 μg/L for waste water (WHO, 1998). The MCL for total cyanide is much higher – 1 mg/L. The group of WAD cyanide has been a subject of special consideration as the assessment of environmental risk and efficiency of detoxification procedures depend on its analytical quantification.

The facts mentioned above highlight the main demands to cyanide determination methods in environmental objects: (i) high sensitivity to reach the low MLC; (ii) high selectivity to analyze a great variety of matrices; (iii) capability for speciation to quantify toxic cyanides; (iv) implementation in portable analytical devices to allow on-site analysis in real time. In the past few years, a variety of new cyanide sensors and improved cyanide determination methods have been reported. Nevertheless, it is not easy to respond to all of the requirements above.

Recently, a review presenting the available methods for cyanide determination and assessing their flexibility to application in automated portable analyzers has been published (Surleva, 2009). The potential of electrochemical detection is specially emphasized in view of its suitability for automation and miniaturization. In portable devices the amperomeric detection has been given preference regardless its low selectivity, which calls for cyanide separation and an on-line method by flow-injection, ligand exchange, and amperometric detection has been officially approved (U.S. EPA, 2004). New flow-injection cyanide selective detectors obtained by thin-layer electrochemical deposition technique have been recently proposed (Neshkova *et al*., 2006; Surleva *et al.*, 2007; Surleva & Neshkova, 2008). The sensors are fully competitive with amperometric detection as far as the lower linear limit, sample throughput, and sensitivity are concerned. Moreover, the potentiometric detectors offer additional advantages: selective response (so that the separation step could be omitted and thus the equipment simplified) and cyanide speciation. Due to the high sensitivity of UV-Vis spectroscopy a lot of research was done in attempt to improve selectivity, analysis time or to develop environmentally friendly procedures. A comparative study of some new and some established spectrophotometric assays for environmental cyanide was reported by Drochioiu *et al.* (2008a; 2011): (i) the Aldridge method and its variants with pyridine and pyrazolone; (ii) isonicotinate-barbiturate method that was useful to detect minute amounts of cyanide *in vivo* and *in vitro*; (iii) the reaction of cyanide ion with ninhydrin, which was proved to be fast, simple, highly selective, and free from most interference, but under reducing conditions; (iv) picric acid-based assay which was described to be highly selective, but yet less sensitive; (v) combined resorcinol-picric acid method which showed improved sensitivity. The achievements in cyanide determination have been recently reviewed by Ma & Dasgupta (2010). This review presented more than 80 papers published between 2005 and 2009. Although the authors claimed to review all the literature published during that period, it seems that they are specially focused on optical detection techniques. Nevertheless, we present here the new articles published from 2009 to 2012 (Table 1). It is worthy to be mentioned the intense research aimed at development of combined colorimetric and fluorescent probes capable to work in 100% aqueous media. Although a lot of work has to be done to propose a robust method, these sensors show very low detection limit coupled with good selectivity, small sample volumes and rapid response. They work on "turn-off and–on" principle and are extremely suitable for portable signaling devises in dangerous environment.

Table 1. Analytical methods for cyanide determination in environmental samples, published between 2010 and April 2012 (Science direct and Springer link data bases)

method	LOD, µg/mL	range, µg/mL	RSD, %	recovery, %	analysis time	object	comments	references
spectrophotometry	0.007	0.01 – 0.5	2-4	97-109	4	tap, mineral and waste water	kinetic mode without separation	Abbasi *et al.*, 2010
spectrophotometry	0.11	0.26–6.5	2	-	7-10	drinking water	optical membrane sensor	Absalan *et al.*,2010
silver nitrate titrimetry	-	-	-	-	-	gold cyanidation solutions	potentiometric and rhodanine end-points	Breuer *et al.*, 2011
conductometry impedance spectroscopy	0.16	up to 1.3	-	-	-	-	catalase biosensor	Bouyahia *et al.*, 2011
voltammetry	0.11	0 – 0.26	-	-	-	-	cytochrome *c* biosensor	Fuku *et al.*, 2012
spectrophotometry	0.16	0.05–2.0	2.3	99-109	5	tap and drinking water	β-correction is used to improve sensitivity	Hamza *et al.*, 2010
spectrophotometry naked eye detection	0.03	-	4-8	95-105	10	drinking water	water soluble chemosensor	Isaad *et al.*, 2011[a] 2011[B], 2011[c], 2010
cuvetteless microspectrophotometry	0.004	0.03 – 0.5	3.9	97	8	river, lake and tap water	headspace single-drop microextraction	Jain *et al*., 2010
spectrophotometry gold nanoparticles	-	down to 0.26	-	-	-	-	AuNPs /Cu^{2+}–phenanthroline sensor	Kim *et al.*, 2010
spectrofluorimetry naked eye detection	0.52	-	-	103	-	drinking water	boronic acid-fluorescein sensor/Gd^{3+} nanoparticles	Kulchat *et al.*, 2012
spectrofluorimetry naked eye detection	0.008	0.5-4.7	2	99	30	drinking water	coumarin-based sensor; mixed solvent	Li *et al.*, 2011
spectrophotometry naked eye detection	0.03	-	-	-	-	-	DMSO/H_2O mixture, thiourea derivatives based sensor	Lin *et al.*, 2011
spectrofluorimetry naked eye detection	0.001	0.01-0.08	-	-	-	-	rhodafluor-based sensor MeOH–H_2O solvent	Lv *et al.*, 2011
voltammetry	0.002	0.001 - 3.9	1.4	98-104	-	industrial wastewater	modified glassy carbon electrode/ Ag nanoparticles	Noroozifar *et al*., 2011
spectrophotometry naked eye detection	-	-	-	-	-	-	thiourea based sensor non-aqueous medium	Odago *et al.*, 2010

spectrophotometry spectrofluorimetry	0.06	-	-	-	-	-	coumarin-based sensor solvent (DMSO/ H_2O)	Park & Kim, 2012
spectrophotometry	0.13	0.13-0.4	-	-	5	-	fluoresceine-spiropyran conjugate; solvent H_2O/MeCN	Sumiya *et al.*, 2012
differential electrolytic potentiometry	-	-	1.4	-	3	water	sequential injection titration	Saleh & Abulkibash, 2011
spectrophotometry spectrofluorimetry	0.039	0.13-1.3	-	-	-		dual colorimetric-fluorescent probe solvent EtOH/H_2O	Tsui *et al.*, 2012
voltammetry	0.0006	0.002–0.08	2 -5	98-102	-	tap water; river water	nanowires array biosensor acid distillation and alkali absorption of HCN	Wang *et al.* , 2010
automatic biodetector coupled with oxygen electrode	0.005	0.001-0.01	-	-	-	water	general estimation of toxicity of water	Woznica *et al.*, 2010
spectrophotometry spectrofluorimetry	-	-	-	-	-	-	naphthalimide based sensor; 100% aqueous medium	Xu *et al.*, 2010
Raman scattering spectroscopy	0.031	0.04 - 4	-	-	5	-	evaporated CuI thin film substrate	Yan et al., 2010
ion-selective potentiometry	0.0003	0.0005-2600	3	102	-	electroplating & photographic wastes; tap water	silver-filled carbon nanotubes	Yari & Sepahvand, 2011
spectrofluorimetry	-	-	-	-	-	-	solvent CH_3CN-H_2O	Yu *et al*., 2010
ion chromatography/ amperometric detection	0.003	0.015 – 2.5	5.20	94-101	25	cigarette main stream smoke	NaOH-treated Cambridge filter for HCN absorption	Zhang *et al.*, 2011
spectrophotometry spectrofluorimetry	0.005	-	-	-	1	-	non-aqueous media	Zhou *et al.*, 2012

b) Cyanide determination in biological samples

Human fluids content cyanide due to different sources of cyanide exposure. Apart from sodium nitroprusside therapy (as a hypotensive agent) and ingestion of cyanide salt in the context of suicidal or homicidal attempts, the main sources of exposure are smoke from fires or cigarette smoking, accidental inhalation of hydrocyanic acid in the metal and plastic industries, and ingestion of various types of food such as cassava, cherry, or almond. Blood cyanide concentration is essential information in medicine and forensic science. Although the state of the objects for analysis is completely different, medical and forensic cyanide analyses have the same difficulties:

(i) First, sample storage and pretreatment significantly affect the results of the analysis. Prior to detection, cyanide needs separation from hemoglobin. This separation is most commonly performed by

acidification using microdifusion in a Conway cell or nitrogen carrying into an alkaline trap solution. The acidification process is prone to errors due to incomplete releasing or artificial cyanide production.

(ii) Second, standard methods for cyanide determination in blood are time consuming and cannot provide adequate data on real time basis. Many of the methods described in the literature are highly sensitive but do not have upper calibration limits high enough to be used in cyanide fatalities. Besides cyanide assay has to differentiate between bound and unbound cyanide to provide data for cyanide antidotes administration.

The postmortem specimens most frequently analyzed for cyanide in forensic toxicology are blood, spleen, liver, and brain. Blood cyanide concentrations lower than 0.25 μg⁄mL are considered normal, and those between 0.25 and 2–3 μg/mL as elevated, but not ordinarily causing death. Concentrations above 3 μg⁄mL are consistent with death in the absence of other relevant or toxicological findings (Gambaro *et al.*, 2007). Animal tissues are other forensic targets for analyzing, especially when illegal use of cyanide compounds in the environment is concerned (Mak *et al.*, 2005a). Therefore, cyanide determination in forensic analysis and cyanide monitoring at very low levels are of great importance (Meng *et al*., 2009).

The analytical techniques for cyanide detection in blood published before 2004 have been critically reviewed by Lindsay *et al*. (2004). Here we present the latest achievements in cyanide determination in biological samples reported after 2004 (Table 2).

In attempt to improve efficiency and accuracy of the sample pre-treatment procedures a hollow fiber-protected headspace liquid-phase microextraction, a headspace single-drop microextraction or solid-supported liquid-liquid extraction combined either with capillary electrophoresis or chromatographic separation were proposed. Interesting approach for cyanide liberation without acidification is an enzymatic degradation of free and complexed cyanide (Mak *et al.*, 2005a,b).

Another research direction is aimed at the development of sensitive and selective detection systems. The lowest detection limit of 0.3 ng/mL was reported for capillary electrophorese with UV detection (Meng *et al.*, 2009).

The widest linear concentration range is reported for gas chromatography/mass spectrometry: 0.05 - 10 μg/mL (Frison *et al*., 2006) and 0.1–20 μg/mL (Liu *et al.*, 2009).

A high selective nafion-modified electrochemical sensor for cyanide determination at physiological pH without separation was described by Lindsay & O'Hare (2006), but additional validation in blood samples is needed. Cyanide instability in post-mortem blood samples was studied and sodium fluoride was proposed to be added to blood samples obtained from fire victims to reduce cyanide instability due to bacteriological activity (McAllister *et al.*, 2011).

Table 2. Analytical methods for cyanide determination in biological samples reported between 2004 and April 2012 (Science direct and Springer link data bases)

method	LOD, µg/mL	range, µg/mL	RSD, %	recovery, %	object	comments	references
spectrofluorimetry spectrophotometry	0.26	0.39 - 2.2	-	-	-	ratiometric and lifetime based sensing	Badugu *et al.*, 2004a; 2004b
gas chromatography/ nitrogen phosphorus detection	0.003	-	12	-	whole blood (mice)	headspace solid-phase micro-extraction	Boadas-Vaello *et al.*, 2008
gas chromatography/ electron capture detector	0.01	0.01 - 0.2 0.2 - 1.0	3 - 7	84–96	whole blood	headspace extraction	Felby, 2009
gas chromatography/ mass spectrometry	0.006	0.05 - 10	8	80	human whole blood	solid-phase microextraction	Frison *et al.*, 2006
spectrophotometry	0.2	0.5 - 10	8	-	post-mortem blood samples	Conway microdiffusion cell	Gambaro *et al.*, 2007
gas chromatography/ nitrogen-phosphorus detector	0.05	0.05 - 5	14	91	post-mortem blood samples	automated headspace extraction	Gambaro *et al.*, 2007
capillary electrophoresis/ UV-spectrometry	0.002	0.007 - 0.52	-	92 - 106	human saliva and urine samples	headspace single-drop microextraction	Jermak *et al.*, 2006
amperometry	0.1	up to 1.3	-	-	blood of burnt victims	nafion-membrane coated electrode	Lindsay & O'Hare, 2006
gas chromatography/ mass spectrometry	0.04	0.1–20	7	91 - 116	plasma and urine	solid-supported liquid–liquid extraction	Liu *et al.*, 2009
flow injection chemiluminescence	0.019	0.013 - 1.3	2	98	rabbit whole blood	fluidic chip design acid distillation	Lv *et al.*, 2005
electrochemical biosensor	0.18	0.78 - 7.8	-	-	fish tissue	enzymatic degradation of free and complexed cyanide	Mak *et al.*, 2005a
spectrophotometry indirect	0.029	0.26 - 2.6			fish tissue	enzymatic degradation of free and complexed cyanide	Mak *et al.*, 2005b
capillary electrophoresis/ UV spectrophotometry	0.0003	0.003 - 0.52	6	92 - 103	non-smokers' and smokers' urine and saliva	hollow fiber-protected headspace liquid-phase micro extraction	Meng *et al.*, 2009
electrospray ionization tandem mass spectrometry	0.001	0.003 - 1.3	13	96 - 117	victim's urine gastric content blood	CN^- + $NaAuCl_4$ to produce dicyanogold, $Au(CN)_2^-$ followed by extraction	Minakata et al., 2009, 2011
capillary electrophoresis/ UV spectrophotometry	0.08	0.4 - 13	3	93 - 106	lysed erythrocyte samples	in-capillary enzymatic reaction of CN^- with rhodanese	Papezova & Glatz, 2006

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gas chromatography–mass spectrometry	0.003	0.026 2.6	-	-	-	smoker and non-smoker plasma	indirect determination of cyanide exposure	Youso *et al.*, 2010
isotope ratio mass spectrometry	-	-		1-7	94 - 105	food, drink, medicine	identifying the origin of cyanide	Tea *et al.*, 2012

c) Cyanide determination in plants

The human's health authorities pay special attention on cyanogens as toxic food constituents, as some cyanogenic plants are staple food in some countries and the population is exposed to high level risk of cyanide intoxication. The analysis of plant and the estimation of its cyanogen content have specific problems related to the need of: (i) hydrolysis and separation of cyanogens or produced cyanide from complex matrices, and (ii) sensitive detection systems. Most of the cyanide related diseases are reported in developing countries, so the availability of the analytical devises to small plants farms is of special importance.

Some summaries of the methods for cyanogenic glycoside determination (although not exhaustive ones) can be found in Herchi *et al.* (2012), Ganjewala *et al.* (2010) and Bjarnholt *et al.* (2008). A review of the recent methods for determination of cyanogens, published between 2000 and 2012 is presented in Table 3.

Table 3. Methods for determination of cyanogenic glycosides in plants and cyanide in foods published from 2000 to April 2012 (Science direct and Springer link data bases)

Method	matrix/sample	cyanogenic compounds	analyte	sample pretreatment	note	references
GC spectrophotometry	sorghum; sudangrass; forage	dhurrin	total cyanide	hydrolysis (121 ^{o}C), liquid and solid phase extraction		Goff *et al.*, 2011
spectrophotometry enzymatic assay	cassava root	amygdalin linumarin	total cyanide	alkaline extraction; enzymatic hydrolysis	range 0.08-2.6µg/mL test plates	Tatsuma *et al.*, 2000
picrate sheet assay solid state detection	-	amygdalin linumarin	total cyanide	enzymatic hydrolysis (pH 6-8) headspace extraction	non-linear response test plates	Abban *et al.*,2011
GC-electron capture/photoionization detection	cassava leaves clover eucalyptus	linamarin lotaustralin prunasin	total cyanide	headspace extraction	LOD 69 ng/mL	Curtis *et al.*, 2002
spectrophotometry Chloramin T/ barbituric/ isonicotinic acids	cassava roots	linamarin lotaustralin	total cyanide	enzymatic hydrolysis (phosphate pH 7); 30 ^{o}C; 15'		Saka & Nyirenda, 2012
Spectro-photometry picrate paper	cassava leaves	linamarin lotaustralin	total cyanide	enzymatic hydrolysis, phosphate buffer (pH 6,5)		Bradbury & Denton, 2011
Spectrophotometry resorcinol/picra	flax seed, stones of peach, plum, nectarine,	amygdalin linustatin neolinustatin	total cyanide	enzymatic hydrolysis; pH 10 ; 16 h; 30 ^{o}C $NaHCO_3$ extraction	LOD 0.05 µg/mL range: 0–5	Drochioiu *et al.*, 2008

te method	apricot, apple seeds				µg/mL ε 9 x 10^3 L/mol·cm	
spectrophotometry picrate method	cassava flour	linumarin	total cyanide	enzymatic hydrolysis	range 0.1- 50 µg/mL	Bradbury, 2009
spectrophotometry ninhydrin method	almond, apple seed, flaxseed, plum kernels	amygdalin linustatin neolinustatin	total cyanide	enzymatic hydrolysis, $NaHCO_3$ extraction, microdiffusion separation	LOD 8 ng/mL range: 0.02-1.0µg/mL ε $1.4x10^5$ L/mol·cm	Surleva & Drochioiu, 2012
spectrophotometry picrate method	cassava, flax seed, sorghum, giant taro leaves, stones of peach, plum, nectarine, apricot, apple seeds bamboo shoot	linumarin dhurrin amygdalin linustatin neolinustatin triglochinin taxiphyllin prunasin	total cyanide	enzymatic hydrolysis in picrate kit; acid hydrolysis	Acid hydrolysis: loss of HCN needs of extrapolation. enzymatic hydrolysis: recovery 101.9 % (S.D. 0.64)	Haque & Bradbury, 2002
electrochemical hydroxyapatite nanowires array biosensor	distilled wine, cassava starch	-	total cyanide	acid distillation and alkali absorption of HCN	LOD 0.6 ng/mL range 2–80 ng/mL	Wang *et al.*, 2010
FIA amperometric biosensor	plant extract	-	total cyanide	batch extraction, enzymatic hydrolysis	LOD 18 ng/mL range 0.02-21 µg/mL	Ketterer& Keusgen, 2010
LC–MS/MS	grapevine cultivars	prunasin sambunigrin	total cyanide	enzymatic hydrolysis		Franks *et al.*, 2005
HPLC	cassava root	linamarin	linamarin	acid extraction with H_2SO_4		Sornyotha *et al.*, 2007
HPLC	sorghum; sudangrass	dhurrin	dhurrin	methanol extraction		De Nicola *et al.*, 2011
GC	flaxseed	linustatin neolinustatin	linustatin neolinustatin	methanol and ethanol extraction	sub-nanogram	Bacala & Barthet, 2007 Barthet & Bacala, 2010

GC- gas chromatography; LC – liquid chromatography; MS – mass spectrometry; FIA – flow injection analysis.

The main trends in the research on cyanogen determination could be summarized as: (i) development of sample pre-treatment procedure suitable for large range of matrices and a great number of cyanogens; (ii) development of efficient cyanide liberation and separation procedures; (iii) development of sensitive and selective detection systems suitable for analyzing small quantities of samples; (iv) development of low cost and easy to maintain equipment.

Cyanogenic glycosides can be determined directly by various chromatographic methods (Table 3 and Herchi *et al.*, 2012, Ganjewala *et al.*, 2010, Bjarnholt *et al.*, 2008). An advantage of chromatographic method is the quantification of cyanogenic glycosides in their native form. Its wide application is limited for a lack of cyanogenic glycoside standards or their high cost.

Indirect cyanogenic glycosides determination, also referred as determination of the plant cyanogenic potential, is based on quantification of HCN released after acidic or enzymatic

hydrolysis of cyanogen glycosides (Table 3).

Efficient extraction and complete hydrolysis is the key for accurate determination of plant cyanogens. Spectrophotometric detection after different color formation reactions is the most widely used in total cyanogens determination: picrate paper assay (Bradbury & Denton, 2011; Bradbury, 2009; Burns *et al.*, 2012), picrate based solid state detection (Abban *et al.*, 2011; Brimer *et al.*, 1998; Haque & Bradbury, 2002); combined picrate/resorcinol method (Drochioiu *et al.*, 2008b), chloramine T/barbituric acid/isonicotinic acid method (Saka & Nyirenda, 2012). Recently, the nynhidrin based method has specially modified for determination of total cyanogens in plants (Surleva & Drochioiu, 2012). A spontaneous enzymatic hydrolysis (at pH 6-8) was combined with extraction using bicarbonate solution or microdiffusion separation. The method is fast, cheap and environmentally friendly. Non-toxic reagents have been used. No special training or sophisticated instrumentation was needed.

Conclusions

This review provides a good example of how the demands of ecology, forensic science and medicine motivate the research and development of new analytical methods and instrumentation.

Rapid cyanide analysis in blood or breath is ripe for new attractive approaches. There are fast acting antidotes for cyanide poisoning, whether from smoke inhalation or exposure to a weapon of terrorism. It is vital to determine blood or breathe cyanide levels fast and accurately so that an appropriate dose of the antidote can be readily determined. Physiological half-life of free cyanide is short and concentration can be affected by storage conditions and many other factors. It is crucial to rapidly analyze such samples, if it possible *in situ*.

The same demand is imposed also by ecology. Due to different toxicity of industrial cyanide containing pollutants, different detoxification procedures have to be applied so that the ecological equilibrium will not be disturbed at a large scale.

Quickly available and highly reliable information about cyanide contamination is required for this purpose.

Because of the importance for clinical, forensic and very likely, security and antiterrorism applications, it has become urgent to establish rapid, sensitive, specific and robust „point of care" cyanide analyzers.

The new colorimetric/fluorimetric probes working on "turn-of-and-on" principle have a lot of promise to be used in small alarm devices or spot tests.

However, a lot of research is needed to validate them in real samples, e.g., air, natural waters, industrial wastewater, biological fluids like urine, blood, saliva etc.

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* Corresponding author: asurleva@yahoo.com