

# Cyanide toxicity of freshly prepared smoothies and juices frequently consumed in the raw and natural food craze

## ABSTRACT

**Aims:** This study was conducted to detect the presence of cyanide in popular fruit and vegetable smoothies and juices marketed as eat raw and natural.

**Study Design:** Eleven (11) popular varieties of drinks were analyzed for total  $\text{CN}^-$  ( $\tau\text{CN}$ ) concentration. Drinks contained raw flax seeds, whole apples with seeds, raw almond milks or, pasteurized almond milk as specified in ingredients.

**Place and Study duration:** Samples were collected from health food eateries located within Las Vegas, Nevada during the summer of 2017.

**Methodology:** Fifty milliliters (mL) of a homogenized smoothie and juice drink and 1 gram of flax seeds were subjected to the above referenced methods for sample preparation per USEPA Methods 9012B (digestion). Samples were then analyzed by USEPA method 9014 (colorimetry).

**Results:** The highest  $\tau\text{CN}$  was detected in drinks containing raw flax seed followed by unpasteurized raw almond milk, then fresh whole apple juice. No  $\tau\text{CN}$  was observed in drinks that contained none of the previous mentioned items (e.g. flax seed, raw almond milk) or those utilizing pasteurized almond milk.

**Conclusion:** This study observed that  $\tau\text{CN}$  is present in smoothies and juices containing raw flax seeds, fresh whole apples, and/or unpasteurized almond milk. Concentrations were detected as high as  $341 \mu\text{g L}^{-1}$  in commercially available smoothies containing vegetables, raw flax seeds, almond milk and fruits. Smoothies with vegetables, fruits, unpasteurized almond milk, and no flax seeds contained  $41 \text{ mg L}^{-1} \text{CN}^-$ , while similar smoothies with pasteurized almond milk contained negligible to  $9.6 \text{ mg L}^{-1} \text{CN}^-$ . Unpasteurized almond milk and raw flax seeds were the major sources of  $\text{CN}^-$ . With the increased demand for raw and natural foods, there is a potential sublethal exposure of  $\text{CN}^-$  by consumers.

*Key words: Cyanide, Smoothie, Juice, amygdalin, linamarin, flax seed, almonds*

31

32 **1. INTRODUCTION**

33 The dangers of  $\text{CN}^-$  to living organisms is well documented; yet, it is still used in various applications  
34 including polymer synthesis, metallurgy, extraction of precious metals, and commercial herbicides  
35 [1,2,3]. It is also found in many plant foods, fruits, and kernels (i.e., seeds) in the form of cyanogenic  
36 glycosides ( $\alpha$ -glycosides or  $\beta$ -glycosides), which are secondary metabolites consisting of  $\alpha$ -  
37 hydroxynitrile and a sugar derivative [4,5]. While anions are known to play a vital role in the  
38 environmental and biological processes of living organisms,  $\text{CN}^-$  derived from cyanogenic glycosides  
39 are toxic and can be damaging to organisms when consumed in large enough quantities [4,6,7]. Lee  
40 et al., [5] and others have shown that anionic species (cyanogenic glycosides as  $\text{CN}^-$ ) are greater in  
41 size with diverse shapes containing higher hydration energies within a wide-ranging scale of  
42 hydrophobicity, ultimately impacting living organisms [4].

43

44 It is known that humans are exposed to low levels of  $\text{CN}^-$  daily from vehicle exhaust, water sources,  
45 foods, and smoking cigarettes [4]. The United States Environmental Protection Agency (USEPA) has  
46 set a Maximum Contaminant Level (MCL) for  $\text{CN}^-$  ( $0.2 \text{ mg L}^{-1}$ ) in drinking water due to evidence  
47 showing nerve and thyroid damage [8]. It is estimated that the general nonsmoking, nonurban  
48 population in the United States is exposed to  $3.8 \text{ } \mu\text{g CN}^-$  per day from atmospheric sources and  $0.4$ -  
49  $0.7 \mu\text{g CN}^-$  found in well-water, assuming consumption of  $\sim 2$  liters per day [9]. Exposure also comes  
50 from naturally occurring cyanogenic glycosides or cyanoglycosides found in foods [10,11].

51

52 Other researchers have identified at least 55 different cyanogenic glycosides in over 2,650 plant  
53 species, many of them used in everyday foods [9,10]. Cyanide in such food items is known to be a  
54 metabolic product of bacteria, fungi, and algae. Many plant species of beans, fruits (e.g. apple and  
55 cherry seeds, almonds, cashew), and flax seed (*Linum usitatissimum*) contain various forms and  
56 concentrations of  $\text{CN}^-$  [11,12,13]. Due to the plethora of  $\text{CN}^-$  containing plants, the World Health  
57 Organization (WHO) has been unable to estimate the total amount of  $\text{CN}^-$  consumed on an average  
58 day per person [14].

59

Raw and natural foods such as seeds and nuts have become a large part of human nutrition with the “eat raw and natural” push over the past decade with a reported 40% of adults consuming raw seeds and nuts daily [4,15]. Most forms of  $CN^-$  in health foods originate from amygdalin (Fig. 1a) contained in apple seeds and almonds or, linamarin (Fig. 1b) contained in flax seeds [4]. Many of these items are used in the health food industry (e.g., fresh smoothies and juices) as a selling point for improving one’s fitness, vigor, and strength as they are a good source of omega-3 fatty acids, lignans, and fiber [4]. Other promotional advantages of these items put into smoothies and juices include weight loss, and improved skin and hair, resulting in an increase of more than 80% in the past 5 years [15]. Moreover, most of these diets have a low protein component which increases the risk of chronic, sublethal  $CN^-$  related effects according to Bolarinwa et al. [4] and others [16]. With this increased demand for these health drinks and additives such as flax seeds added for their omega-3 fatty acids, antioxidants, and fiber, there is a potential for sublethal exposure to  $CN^-$ , leading to a variety of chronic health related consequences [17].

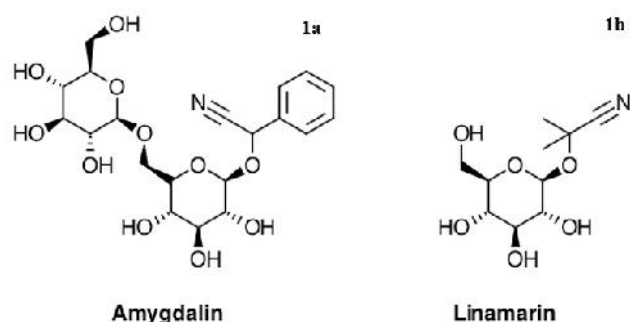


Figure 1a: Cyanide form (“Amygdalin”) found in apple seeds and almonds; 1b: Cyanide form (“Linamarin”) found in flax seeds.

### 1.1 Cyanide Toxicity

When exposed to cyanogenic glycosides, an organism’s breakdown of  $CN^-$  is dependent on the presence and amount of hydrolytic enzymes [18,19,20]. Other authors have shown that when there are not enough hydrolytic enzymes to break down  $CN^-$ , a lethal (“oral”) dose of  $CN^-$ , or acute toxicity, can be as much as  $0.54 \text{ mg } CN^- \text{ kg}^{-1}$  of body weight [9]. Studies have illustrated that  $CN^-$  ranging between  $0.5$  and  $3.5 \text{ mg } \text{kg}^{-1}$  of body weight can also lead to acute poisoning [4]. Its ( $CN^-$ ) affect works by binding to the trivalent iron found in cytochrome c of the oxidative phosphorylation pathway, preventing the cell from utilizing oxygen, resulting in cellular hypoxia and decreased adenosine

triphosphate (ATP) production [9,16]. Cyanide forces cells into anaerobic metabolism to produce ATP, resulting in a buildup of lactic acid, leading to an acid-base imbalance causing metabolic acidosis [21,22]. In addition to binding with cytochrome c,  $CN^-$  inhibits free radical scavengers such as catalase and peroxidase, producing further oxidative stress, phosphatase preventing ATP production, and succinic dehydrogenase reducing the production of CoQ10 that is needed for producing cellular energy and removing free radicals [21,23].

The primary targets for  $CN^-$  toxicity are the cardiovascular, respiratory, and central nervous systems [24]. The central nervous system, especially the brain, is the most significantly affected [21]. Studies of populations that rely primarily on cassava for dietary starches have shown that chronic consumption can lead to sublethal  $CN^-$  exposure. This exposure can result in severe neurologic effects such as hyperreflexia or spastic paraparesis of the extremities known as Konzo, spastic dysarthria, visual and hearing difficulties, cerebellar signs, and memory impairment [17,21,23,25,26,27].

Soto-Blanco et al. [20] explained that  $CN^-$  is ubiquitous in the environment and when exposed to unborn goat fetus there are embryo-toxic and teratogenic effects however, the  $LD_{50}$  for  $CN^-$  exposure was established in animal studies and is insufficient to provide a full assessment of risk to human fetuses. Moreover, due to complications related to  $CN^-$  poisoning and the lack of data concerning the teratogenicity of  $CN^-$ , it is not currently possible to state that an absence or presence of maternal  $CN^-$  exposure excludes any adverse effects to the development of a human fetus or during breast feeding. However, animal studies clearly showed that fetal effects did exist between mother and fetus due to the transfer of  $CN^-$  during the pregnancy [20]. Finally, Soto-Blanco and Gorniak [18] also showed that there is a transfer of  $CN^-$  from mother to goats (kids) through suckling (breast feeding) of milk.

The popularity of raw almonds, flax seeds and other foods deemed as healthy has been rising with little to no oversight by regulatory agencies. Cyanide toxicity in foods is not a new issue; it has been documented that people have died due to consuming raw almonds in excess [12,28]. The purpose of the present study was to examine  $CN^-$  levels within health food drinks (i.e., fresh smoothies and juices) being promoted as healthy.

This study evaluated the potential exposure of CN<sup>-</sup> by consumers drinking freshly prepared smoothie and juice drinks purchased at health food eateries in Las Vegas, Nevada (USA). Samples were collected from popular eateries and analyzed for total CN<sup>-</sup> utilizing USEPA approved methods. The primary goals of this study were to 1) evaluate smoothies for CN<sup>-</sup> content by analyzing samples within 1 hour of preparation; 2) discuss CN<sup>-</sup> source in beverage components; and 3) discuss exposure to an average person consuming such beverages.

## 2. MATERIALS AND METHODS

Raw and fresh drinks (i.e. smoothie and juices) are made with whole fruits (i.e. apples), almond milk, protein alternatives, seeds, and other items deemed healthy alternatives. Drinks were purchased locally from a variety of health food eateries within Las Vegas, Nevada between May and July 2017. A total of eleven (11) popular varieties of drinks were analyzed for total CN<sup>-</sup> (τCN) concentration (Table 1a) over this period. Drinks contained either raw flax seeds, whole apples with seeds, raw almond milks or, pasteurized almond milk as specified in ingredients. Since flax seeds are an additive for their reported health benefits, brown and yellow flax seeds (smoothie additive) were collected from smoothie eateries and analyzed for τCN (Table 1b). The intent behind testing flax seed separately was to compare τCN concentrations detected in drinks with and without flax seeds. Apple seeds were not analyzed separately as they are part of the whole apples and are not additive but rather, part of the apple when juiced. Additionally, two beverages (E1 and F1) were analyzed for τCN that are sold as healthy alternatives to high sugar drinks for children and contain pasteurized apple and apricot juice. These two juice drinks were analyzed to evaluate the effectiveness of pasteurization on natural contaminants.

**Table 1a: Smoothie and juice ingredients per drink**

| Drink Type | ID | Ingredients                                                                                                                                                                                  |
|------------|----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Smoothie   | A1 | Pasteurized almond milk, cocoa powder, dark chocolate chips, coffee beads, avocado, spinach, kale, collard greens, raw flax seed, raisins, whole red/green apple, vanilla, agave nectar, ice |
| Smoothie   | A2 | Cocoa powder, dark chocolate chips, banana, roasted peanuts, avocado, kale, spinach, raw flax seed, zucchini, chia, honey, vanilla, agave nectar, ice                                        |
| Smoothie   | A3 | Green grapes, whole green apple, pineapple, orange, lime, wheat grass, kale, spinach, collard greens, ginger, raw flax seed, agave nectar, ice                                               |
| Smoothie   | B1 | Strawberries, blueberries, cranberry, multi-vitamin, raw ground flax                                                                                                                         |

|          |    |                                                                                                |
|----------|----|------------------------------------------------------------------------------------------------|
|          |    | seed, whole grain oats, whey protein, Splenda, ice                                             |
| Smoothie | B2 | Blueberries, mango, banana, roasted almonds, protein, ice                                      |
| Smoothie | C  | Unpasteurized almond milk, spinach, banana, vanilla, syntha-6, soy protein, yogurt, ice        |
| Smoothie | D1 | Cucumber, fresh apples, spinach, grapes, Greek yogurt, roasted pumpkin seeds, lemon juice, ice |
| Smoothie | D2 | Whole apples, pineapple, kale, spinach, chia seed, ice                                         |
| Juice    | E1 | Packaged, pasteurized apple juice for babies                                                   |
| Juice    | E2 | Freshly juiced whole apples                                                                    |
| Juice    | F1 | Packaged, pasteurized apricot juice                                                            |

**Table 1b: Raw flax seeds**

| Seed             | ID | Type   |
|------------------|----|--------|
| Flax seed, whole | G1 | Brown  |
| Flax seed, whole | G2 | Yellow |

Drinks and flax seeds were analyzed per USEPA Methods 9012B (digestion) and 9014; distillation was followed by colorimetry. Fifty milliliters (mL) of each smoothie and juice drink (homogenized) along with 1 gram of flax seeds were subjected to the above referenced methods for analysis. The method (9012B and 9014) was modified for this study with an additional 90 minutes for digestion and distillation plus another 15 minutes for cool down. This method extracts hydrogen  $CN^-$  (i.e., hydrocyanic acid) as  $\tau CN$  from samples with  $H_2SO_4$  followed by absorption of  $\tau CN$  in a gas scrubber using a 0.25N sodium hydroxide solution. A buffer of sodium dihydrogen phosphate ( $1M NaH_2PO_4 \cdot H_2O$ ) was added for stabilization followed by the additions of chloramine-T ( $C_7H_7ClNNaO_2S$ ) and pyridine-barbituric ( $C_5H_5N \cdot C_4H_4N_2O_3$ ) acid to generate color for analysis of  $CN^-$  via a Beckman Coulter UV colorimetry instrument at 578 nm wavelength. Methods utilized in this study were verified for the use of plant material and compared to other methods by Gleadow et al. [29] and by Ketterer and Keusgen [30].

Quality control was performed with two separate sources of solid potassium  $CN^-$  (KCN) purchased from two different USEPA approved vendors. Initial and continuing calibration verifications (Alfa Aesar, lot # 10193459) were performed with acceptable windows of 90-110%. Additionally, a laboratory control sample (LCS) was analyzed (Sigma-Aldrich, lot # MKBZ8253V) with acceptable

windows of 85-115%. Calibration ranged from 0 to 600  $\mu\text{g L}^{-1}$  with a method detection limit (MDL) established at 2  $\mu\text{g L}^{-1}$  for this study per USEPA method 9014. All samples were analyzed in duplicate and digested followed by distillation per the method. Duplicate sample data was expressed as relative percent difference between duplicates (RPD) with acceptable USEPA windows of  $\pm 20\%$  [8].

### 3. RESULTS

Results for 11 popular smoothies and juices and two flax seed additives commonly used in these products are presented in Tables 2 and 3. In order of highest to lowest,  $\tau\text{CN}$  was detected in drinks containing flax seed followed by drinks with unpasteurized raw almond milk, and then fresh whole apple juice. No detectable  $\tau\text{CN}$  was observed in drinks that contained none of the previous mentioned items (e.g. flax seed, raw almond milk) or those that used pasteurized almond milk.

Data shows a wide range of  $\tau\text{CN}$  concentrations across drinks and within a single eatery where drinks were purchased on different days of the week. Freshly made smoothies (ID: B, D, E, F) viewed as providing a healthy alternative contained non-detectable (ND)  $\text{CN}^-$  to as much as 341  $\mu\text{g L}^{-1}$   $\tau\text{CN}$  (ID: A2). Smoothies prepared with flax seeds contained the highest concentrations while smoothies with no flax seeds contained measurable  $\text{CN}^-$ , though significantly lower than those with flax seeds. Freshly juiced apples (E2) contained low concentrations of  $\text{CN}^-$  (2.7  $\mu\text{g L}^{-1}$ ), while prepackaged juices (IDs: E1 and F1) and those made with no flax seeds (B2, D1, D2) contained no detectable  $\tau\text{CN}$ .

**Table 2:  $\text{CN}^-$  concentration of juices and smoothies commonly consumed**

| Drink Type | Product / Type                                                                                                                                                                         | ID  | $\tau\text{CN}$<br>$\mu\text{g L}^{-1} \pm \text{RPD}$ |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|--------------------------------------------------------|
| Smoothie   | Pasteurized almond milk, cocoa powder, dark chocolate chips, coffee beads, avocado, spinach, kale, collard greens, raw flax seed, raisins, whole red apple, vanilla, agave nectar, ice | A1a | 272 $\pm$ 17                                           |
| Smoothie   | Pasteurized almond milk, cocoa powder, dark chocolate chips, coffee beads, avocado, spinach, kale, collard greens, raw flax seed, raisins, whole red apple, vanilla, agave nectar, ice | A1b | 134 $\pm$ 0.1                                          |

|          |                                                                                                                                                                         |     |           |
|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|-----------|
| Smoothie | Pasteurized almond milk, cocoa powder, dark chocolate chips, coffee beads, avocado, spinach, kale, collard greens, raisins, whole red apple, vanilla, agave nectar, ice | A1c | 9.6 ± 2.4 |
| Smoothie | Cocoa powder, dark chocolate chips, banana, roasted peanuts, avocado, kale, spinach, raw flax seed, zucchini, chia, honey, vanilla, agave nectar, ice                   | A2  | 341 ± 8.1 |
| Smoothie | Green grapes, whole green apple, pineapple, orange, lime, wheat grass, kale, spinach, collard greens, ginger, raw flax seed, agave nectar, ice                          | A3  | 158 ± 0.5 |
| Smoothie | Strawberries, blueberries, cranberry, multi-vitamin, raw ground flax seed, whole grain oats, whey protein, Splenda, ice                                                 | B1  | 205 ± 6.0 |
| Smoothie | Blueberries, mango, banana, roasted almonds, protein, ice                                                                                                               | B2  | ND        |
| Smoothie | Unpasteurized almond milk, spinach, banana, vanilla, syntha-6, soy protein, yogurt, ice                                                                                 | C   | 41 ± 6.5  |
| Smoothie | Cucumber, fresh apples, spinach, grapes, Greek yogurt, roasted pumpkin seeds, lemon juice, ice                                                                          | D1  | ND        |
| Smoothie | Whole apples, pineapple, kale, spinach, chia seed, ice                                                                                                                  | D2  | ND        |
| Juice    | Packaged, pasteurized apple juice for babies                                                                                                                            | E1  | ND        |
| Juice    | Freshly juiced whole apples                                                                                                                                             | E2  | 2.7 ± 0.1 |
| Juice    | Packaged, pasteurized apricot juice                                                                                                                                     | F1  | ND        |

^ (A1b) purchased from the same location as A1a however, purchased one week apart to evaluate consistency in formula. RPD is relative percent difference between duplicates samples. ND: non-detectable

Samples purchased from the same eatery were inconsistent between drink mixtures with smoothie A1a and A1b contained 272 and 134  $\mu\text{g kg}^{-1}$ , respectively. Both smoothies (A1a and A1b) were collected from the same eatery, one-week apart. This difference was likely the result of inconsistency in drink preparation by eateries staff. Samples with flax seeds contained the highest  $\gamma\text{CN}$ , while drinks with no flax seeds contained the lowest; flax seed contains linamarin, a natural source of  $\text{CN}^-$  [4]. Samples having low levels of  $\gamma\text{CN}$  and no flax seed were made from freshly juiced apples with seeds, a source recognized to contain  $\text{CN}^-$  in the form of amygdalin [4]. Both sources of  $\text{CN}^-$  are liberated from apple seeds during the juicing process, leaving detectable levels in drinks.

**Table 3: CN<sup>-</sup> concentration (mg kg<sup>-1</sup>) in flax seeds used as additives to juices and smoothies**

| Seed      | Product    | Type             | ID | τCN<br>mg kg <sup>-1</sup> ± RPD |
|-----------|------------|------------------|----|----------------------------------|
| Flax Seed | Whole seed | Brown Flax Seed  | G1 | 60 ± 1.9                         |
| Flax Seed | Whole seed | Yellow Flax Seed | G2 | 51 ± 4.6                         |

Note: RPD is the relative percent difference between duplicates samples

Flax seed is added to smoothies as either whole or ground seeds for their reported health benefits, blended for homogeneousness to release nutrients, and consumed. Sample G1 (brown flax) contained 60 mg kg<sup>-1</sup>, while sample G2 (yellow flax) contained 51 mg kg<sup>-1</sup> of τCN. It was observed that smoothies prepared with fresh whole apples, and no flax seeds also contain CN<sup>-</sup> (i.e. A1c 9.6 μg L<sup>-1</sup> and E2 2.7 μg L<sup>-1</sup> τCN), though considerably lower than those with flax seeds as illustrated in A1a, A1b, A2, A3, and B1 with 272, 124, 341, 158, and 205 μg L<sup>-1</sup>, respectively. Moreover, smoothies with added flax seeds were 1 order of magnitude higher in τCN than smoothies containing no flax seeds.

#### 4. DISCUSSION

It has been observed that consumption of foods containing CN<sup>-</sup> (e.g. raw almonds) causes intercellular hypoxia when it binds to mitochondrial cytochrome c oxidase a3 after consumption [13]. Eleven (11) freshly prepared smoothies and juices along with 2 flax seed additives were evaluated for total τCN concentrations to determine the risk of this food deemed a healthy alternative to processed foods. Eight (8) of the 11 drinks contained various levels of CN<sup>-</sup> ranging from 2.7 to 341 μg kg<sup>-1</sup> τCN. Flax seeds, a popular health food additive to these drinks, contained between 51 and 60 mg kg<sup>-1</sup> τCN. Studies have shown that ingredients such as apple and flax seeds contain amygdalin and linamarin, two of the most common forms of cyanogenic glycosides found in raw foods [4,15].

Flax seeds are marketed as a super-food promoting fitness, vigor, strength, hair growth and other benefits [4]. Studies suggest that natural foods including flax seeds not be consumed during pregnancy or, breastfeeding, due to possible effects on the unborn baby [31]. These findings show that drinks containing flax seeds had the highest levels of CN<sup>-</sup> ranging from 134 to 341 μg kg<sup>-1</sup> τCN.

Drinks with no flax seeds ranged between ND and  $41 \mu\text{g kg}^{-1}$   $\text{CN}^-$ . Levels of  $\text{CN}^-$  in this study suggest that such consumption during pregnancy or breastfeeding may pose a sublethal exposure to the unborn child.

Linamarin, a cyanogenic glucoside in flax seeds is also in the leaves and roots of plants such as cassava and lima beans [4,15]. Authors describe linamarin as a glucoside of acetone cyanohydrin that when consumed raw, can cause adverse effects on the body due to its  $\text{CN}^-$  content [9]. Other researchers have reported that upon exposure to endogenous  $\beta$ -glycosidase enzyme in the human gut, linamarin and its methylated relative lotaustralin can decompose to HCN, creating a possible avenue to sublethal exposure [27,32,33].

Although most parts of a plant can contain cyanoglycosides, studies have shown it to be concentrated in the seeds [27]. Apple seeds contain amygdalin (cyanogenic glycosides) and when juiced release low levels of  $\text{CN}^-$ . Studies have shown that the seeds of apples contain low levels of  $\text{CN}^-$ , this study showed that drinks with apple seeds and no flax seed contained between ND to  $9.6 \text{ mg L}^{-1}$ . When a drink contained flax seeds  $\text{CN}^-$  levels were significantly higher than those made with just whole apple. It is known that when a drink is blended, the juicing process liberates  $\text{CN}^-$  as a result of hydrolysis upon contact with water and enzymes and, from the vigorous blending and aeration [12,16]. However, the hydrolysis has limited impact with higher concentrations of  $\text{CN}^-$  sources such as raw flax seeds leaving detectable levels. It is also known that microorganisms in the gastrointestinal system also produce the enzymes  $\beta$ -glycosidase and hydroxynitrile lyase that convert glycosides into HCN; another mechanism creating a pathway to exposure [16,27,32].

It is important to point out that linamarin is only partially metabolized (~25%) to HCN with the remaining unchanged linamarin excreted by the kidneys [34]. Animal researchers evaluated dogs and rats by feeding a linamarin (e.g., cassava) diet that led to the development of diabetes mellitus [32,34,35]. They also determined that cyanide from linamarin can cause hypertrophy of the adrenal gland, leading to decreased function and hypoadrenocorticism [27,36]. Cyanide can also impact the adrenal gland function, resulting in weight gain and the risk of developing diabetes mellitus [27]. Therefore, diets focused on weight loss can have the opposite effect, causing the consumer to gain weight as a result of  $\text{CN}^-$  intake.

The metabolic product thiocyanate, while less toxic to the body than  $\tau$ CN, is not completely innocuous. Thiocyanate competes with iodine uptake and utilization by the thyroid gland, leading to the formation of goiters and hypothyroidism [37]. Cliff et al [37] also explain that hypothyroidism leads to weight gain, furthering the effects of  $\tau$ CN on the development of diabetes mellitus. Therefore, people with thyroid disease may experience further problems with their thyroid gland.

A noteworthy concern with linamarin is when there are dietary deficiencies in sulfur containing proteins. The liver enzyme rhodenese, or thiosulfur transferase, uses sulfur to convert  $\tau$ CN to the less toxic thiocyanate that is excreted by the kidneys [16]. Approximately 80% of absorbed  $\tau$ CN metabolized to thiocyanate is excreted from the body within 24 hours according to Carlson [38] with the rate of detoxification by the body estimated to be  $1\mu\text{g kg}^{-1}$  body weight/min [9]. If sulfur is deficient due to diet, the rate of detoxification slows, exacerbating the effects of  $\tau$ CN [16,34,38].

Since  $\text{CN}^-$  is metabolized by the body, the median lethal dose ( $\text{LD}_{50}$ ) is time dependent. This means that the  $\text{LD}_{50}$  is greater for chronic exposure verses acute exposure [21]. Slight effects have been noted with  $\tau$ CN levels ranging between 20 and  $40\mu\text{g kg}^{-1}$  body weight, whereas levels of  $50\text{--}60\mu\text{g kg}^{-1}$  body weight can be tolerated without immediate or later effects between 20 and 60 minutes after exposure [9]. Based on this information, an oral minimum risk level of  $0.05\text{ mg kg}^{-1}$   $\text{CN}^-$  of body weight/day has been established for an intermediate duration of exposure with no long-term exposure limits established [21]. Thus, an average 70 kg adult can consume up to 3.5 mg  $\tau$ CN over the course of a day without developing overt clinical signs of cyanide toxicosis. The highest concentration of  $\tau$ CN in this study contained  $341\mu\text{g L}^{-1}$  (sample A2) in a standard size of 650 mL - equaling 221  $\mu\text{g}$  (0.221 mg  $\tau$ CN per serving) per standard size. An adult would have to consume 16 regular sized smoothies in less than two hours to be lethal [39]. This study is not suggesting that a person can consume a lethal dose through smoothies in two hours but rather, sublethal amounts over an extended period of time.

Another important point to make is when smoothies containing freshly juiced vegetables, fruits and raw seeds (i.e., flax) are consumed by children as young as 3 years of age, they may have a greater risk for a sublethal effect. The average weight of a 3-year-old is 14 kg [37], consuming multiple smoothies equaling 0.221 mg  $\tau$ CN per serving would be enough to cause clinical signs of acute  $\tau$ CN

toxicity (~3 regular sized smoothies). Although the  $\tau$ CN content in a single smoothie (0.221 mg  $\tau$ CN per serving) is below the detoxification level of 1  $\mu\text{g kg}^{-1}$  body weight/min for producing overt clinical signs, researchers have shown that chronic low-level exposure (sublethal) can cause histologic effects leading to clinical problems over the course of time [9,16,36] A single drink will not cause an acute effect to the consumer however, people on a healthy-green diet, those with compromised hepatic systems or, an underdeveloped hepatic system, may experience long-term impacts from the consumption of these types of drinks.

## 5. CONCLUSION

The current study has observed that  $\tau$ CN is present in smoothies and juices containing raw flax seeds, fresh whole apples, and/or unpasteurized almond milk. Potentially any fruit or vegetable containing cyanogenic glycosides, linamarin and amygdalin, may be contributing to  $\tau$ CN content in health food drinks such as smoothies. Cyanide from linamarin has been linked to a variety of health issues such as diabetes mellitus, neurological deficits, sensory or memory impairments, and weight gain through damage to the adrenal gland function. Moreover, thiocyanate, a metabolic by-product of  $\text{CN}^-$ , has been tied to goiter growth and hypothyroidism. The presence of  $\text{CN}^-$  in these drinks do not pose an acute threat of poisoning; however, this study suggests that a diet consisting of regular raw flax seeds, fresh whole apples, and/or unpasteurized almond milk, smoothie intake may result in chronic sublethal exposure to  $\tau$ CN. The average adult can mitigate  $\text{CN}^-$  toxins consumed in their daily diets. Women who may become pregnant, currently pregnant and people with developing or, compromised immune systems should monitor or restrict their intake of drinks containing raw flax seeds and almonds or unpasteurized almond milk. Finally, additional research is required to fully understand the possible health effects that exist in unprocessed fresh foods.

## REFERENCE

1. Dash, R. R., Gaur, A., Balomajumder, C. 2009. Cyanide in industrial wastewaters and its removal: a review on biotreatment, *Journal of Hazardous Materials*, 163, 1–11.
2. Dubey, S. K., Holmes, D. S. 1995. Biological cyanide destruction mediated by microorganisms, *World Journal Microbiology. Biotechnology*, 11, 257–265.
3. Murthy, Z. V. P., Gupta, S. K. 1999. Sodium cyanide separation and parameter estimation for reverse osmosis thin film composite polyamide membrane, *Journal of Membrane Science*, 154, 89–103.
4. Bolarinwa, I., Orfila, C., Morgan, M. 2013. Amygdalin content of seeds, kernels and food products commercially-available in the UK. School of food science and nutrition, University of Leeds LS2 9JT, United Kingdom.
5. Lee, J. Y., Kim, S. K., Jung, J. H., Kim, J. S. 2005. Bifunctional fluorescent calixarenechemosensor for both a cation and an anion, *Journal of Organic Chemistry*, 70, 1463–1466.

6. Park, C. H., Simmons, H. E. 1968. Encapsulation of halide ions by in, in-1,(k + 2)-diazabicyclo[k.l.m.]alkane ammonium ions, *Journal of the American Chemical Society*, 90, 2431–2432.
7. Jaszczak, E., Polkowska, Ż., Narkowicz, S., Namieśnik, J. 2017. Cyanides in the environment—analysis—problems and challenges. *Environmental Science and Pollution Research*, 24(19), DOI 10.1007/s11356-017-9081-7.
8. United States Environmental Protection Agency (USEPA). 2017. Drinking Water Contaminant Human Health Effects Information. Accessed December 15, 2017. <https://www.epa.gov/dwstandardsregulations/drinking-water-contaminant-human-health-effects-information>.
9. Simeonova, F. P. and Fishbein L. 2004. Hydrogen cyanide and cyanides: human health aspects. World Health Organization. Geneva, Switzerland.
10. Cho, E. G., Zaremba J. D., McKercher, S. R. 2011. MEF2C enhances dopaminergic neuron differentiation of human embryonic stem cells in a parkinsonian rat model. *PLoS ONE*, 2011, 6:e24027.
11. Francisco, I. A., Pinotti, M. H. P. 2000. Cyanogenic glycosides in plants. *Brazilian Archives of Biology and Technology*, 43, 487-492.
12. Chaouali, N., Gana, I., Dorra, A., Khelifi, F., Nouioui, A., Masri, W., Belwaer, I., Ghorbel, H., Hedhili, A. 2013. Potential Toxic Levels of Cyanide in Almonds (*Prunus amygdalus*), Apricot Kernels (*Prunus armeniaca*), and Almond Syrup. *Toxicology*, 1-6.
13. Haque, M. R. and Bradbury, J. H. 2002. Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. *Food Chemistry*, 77, 107-114.
14. World Health Organization (WHO). 2004. Changing history. Geneva: 2004, 1–96.
15. Lewis, K. 2005. Persister cells and the riddle of biofilm survival. *Biochemistry (Mosc.)*, 70, 267–274. DOI:10.1007/s10541-005-0111-6.
16. Abraham, K., Buhrke, T. Lampen A. 2016. Bioavailability of cyanide after consumption of a single meal of foods containing high levels of cyanogenic glycosides: a crossover study in humans. *Archives of Toxicology*, 90, 559-574.
17. Keefe, M. H., Benkstein K. D., Mathangi D.C., Namasivayam A. 2000. Effect of chronic cyanide intoxication on memory in albino rats. *Food and Chemical Toxicology*, 38, 51-55.
18. Soto-Blanco, B., Górniak, S. L. 2003. Milk transfer of cyanide and thiocyanate: Cyanide exposure by lactation in goats. *Vet. Res.*, 34: 213-220.
19. Soto-Blanco, B., Stegelmeier, B. L., Pfister, J. A., Gardner, D. R. and Panter, K. E. 2008, Comparative effects of prolonged administration of cyanide, thiocyanate and chokecherry (*Prunus virginiana*) to goats. *J. Appl. Toxicol.*, 28: 356–363. doi:10.1002/jat.1286.
20. Soto-Blanco, B., Pereira, F. T. V. de Carvalho, A. F., Miglino M. A., Górniak, S. L. 2009. Fetal and maternal lesions of cyanide dosing to pregnant goats. *Small Ruminant Res.*, 87: 76-80.
21. Agency for Toxic Substances and Disease Registry (ATSDR). 2006. Toxicological profile for cyanide. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA.
22. New York State Department of Health (NYSDH). 2004. The facts about cyanides. Accessed September 05, 2017. [https://www.health.ny.gov/environmental/emergency/chemical\\_terrorism/cyanide\\_tech.htm](https://www.health.ny.gov/environmental/emergency/chemical_terrorism/cyanide_tech.htm).
23. Sreeja, V. G., Leelamma S. 1996. Effect of protein supplemented cassava diet in rats. *Indian Journal of Biochemistry and Biophysics*, 33,149-151.
24. Sreeja, V. G., Nagahara N., Li Q., Minami M. 2003. New aspects in pathogenesis of konzo: neural cell damage directly caused by linamarin contained in cassava (*Manihot esculenta* Crantz). *British Journal of Nutrition*, 90, 467-472.
25. Osuntokun, B. O., Monekosso G. L., Wilson J. 1969. Relationship of a degenerative tropical neuropathy to diet-report of a field survey. *British Medical Journal*, 1, 547-550.
26. Mathangi D. C., Namasivayam A. 2000. Effect of chronic cyanide intoxication on memory in albino rats. *Food and Chemical Toxicology*, 38, 51-55.
27. Knight, A. P., Walter, R. G. 2001. Plants causing sudden death-plants containing cyanide glycosides. In: Cann CC, eds. A guide to plant poisoning of animals in North America. Jackson, WY. Teton NewMedia; pp. 1-5.
28. Hall, A. H., Rumack, B. H. 1986. Clinical Toxicology of Cyanide. *Annals of Emergency Medicine*, 15(9), 115-122.
29. Gleadow, R. M., Møldrup, M. E., O'Donnell, N. H. and Stuart, P. N. 2012. Drying and processing protocols affect the quantification of cyanogenic glucosides in forage sorghum. *Journal of the Science of Food and Agriculture*, 92, 2234–2238. doi:10.1002/jsfa.5752.

30. Ketterer, L., Keusgen. M. 2010. Amperometric sensor for cyanide utilizing cyanidase and formate dehydrogenase. *Analytica Chimica Acta*, 673, 54-59.
31. Magee, E. 2009. The benefits of flaxseed. WebMD. Accessed October 15, 2016. <https://www.webmd.com/diet/features/benefits-of-flaxseed#1> (accessed November 13, 2017).
32. Kahn, C. M., Line S., eds. 2010. Cyanide poisoning. In: *The Merck Veterinary Manual*, 10<sup>th</sup> edition. Whitehouse Station, NJ. Merck and Company, Inc., pp. 2550-2553.
33. Rivadeneyra-Domínguez, E., Vázquez-Luna A., Rodríguez-Landa J. F., Díaz-Sobac R. 2013. Neurotoxic effect of linamarin in rats associated with cassava (*Manihot esculenta* Crantz) consumption. *Food and Chemical Toxicology*, 59, 230-235.
34. Kamalu, B. P. 1995. The adverse effects of long-term cassava (*Manihot esculenta* Crantz) consumption, *International Journal of Food Sciences and Nutrition*, 46(1), 65-93, DOI: 10.3109/09637489509003387.
35. Akanji, A. O., Famuyiwa O. O. 1993. The effects of chronic cassava consumption, cyanide intoxication and protein malnutrition on glucose tolerance in growing rats. *British Journal Nutrition*, 69, 269-276.
36. Kamalu B. P. 1993. Pathological changes in growing dogs fed on a balanced cassava (*Manihot esculenta* Crantz) diet. *British Journal of Nutrition*, 69, 921-934.
37. Cliff, J., Lundquist P., Rosling H., Sörbo B., Wide L. 1986. Thyroid function in a cassava-eating population affected by epidemic spastic paraparesis. *Acta Endocrinologica (Copenh)*, 113, 523-528.
38. Carlson, M. 1999. Glucose repression in yeast. *Current Opinion in Microbiology*, 2, 02-207. DOI: 10.1016/S1369-5274(99)80035-6.
39. Bhandari, R. K., Oda, R. P., Petrikovics, I., Thompson, D. E., Brenner, M., Mahon, S. B., Bebart, V. S., Rockwood, G. A., Logue, B. A. 2014. Cyanide Toxicokinetics: The Behavior of Cyanide, Thiocyanate and 2-Amino-2-Thiazoline-4- Carboxylic Acid in Multiple Animal Models. *Journal of Analytical Toxicology*, 38, 218-225.