

**Original Research Article**

**Carcinogenic and Neurotoxic Risks of Acrylamide Consumed through Caffeinated Beverages  
among the Lebanese Population**

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## Abstract

The present study aims to quantify acrylamide in caffeinated beverages including American coffee, Lebanese coffee, espresso, instant coffee and hot chocolate, and to determine their carcinogenic and neurotoxic risks. A survey was carried for this purpose whereby 78% of the Lebanese population was found to consume at least one type of caffeinated beverages. Gas Chromatography Mass Spectrometry analysis revealed that the average acrylamide level in caffeinated beverages is 29,176  $\mu\text{g/kg}$  sample. The daily consumption of acrylamide from Lebanese coffee (10.9  $\mu\text{g/kg-bw/day}$ ), hot chocolate (1.2  $\mu\text{g/kg-bw/day}$ ) and Espresso (7.4  $\mu\text{g/kg-bw/day}$ ) was found to be higher than the risk intake for carcinogenicity and neurotoxicity as set by World Health Organization (WHO; 0.3-2  $\mu\text{g/kg-bw/day}$ ) at both the mean (average consumers) and high (high consumers) dietary exposures. On the other hand, American coffee (0.37  $\mu\text{g/kg-bw/day}$ ) was shown to pose no carcinogenic or neurotoxic risks among the Lebanese community for consumers with a mean dietary exposure. The study shows alarming results that call for regulating the caffeinated product industry by setting legislations and standard protocols for product preparation in order to limit the acrylamide content and protect consumers. In order to avoid carcinogenic and neurotoxic risks, we propose that WHO/FAO set acrylamide levels in caffeinated beverages to 7,000  $\mu\text{g}$  acrylamide/kg sample, a value which is 4-folds lower than the average acrylamide levels of 29,176  $\mu\text{g/kg}$  sample found in caffeinated beverages sold in the Lebanese market.

## Key Words

Caffeinated beverages; Lebanese coffee, Espresso; American coffee; acrylamide; carcinogenic; neurotoxic; Gas Chromatography Mass Spectrometry.

## Chemical Compounds Studied in this article

Acrylamide (PubChem CID: 6579)

## 1. Introduction

Coffee products have been shown to possess antioxidant activity and their intake reduces the risk of development of some types of cancer, such as colon cancer (Tavani and La, 2000). This anti-cancer activity can be attributed to caffeine which is the active ingredient in such products and after which the beverage was named (Iwa et al., 2012). According to Iwa et al., chlorogenic acids included in green and roasted coffee beans possess various biological benefits such as antioxidant, radical scavenging, antimutagenic/anticarcinogenic as well as anti-inflammatory activities (Iwa et al., 2012). In addition, coffee consumption has been shown to aid the fight against obesity while also limiting the effects of type II diabetes by decreasing glucose absorption (Bassoli et al., 2008). On the other hand, coffee is a main contributor to the human dietary intake of acrylamide, a chemical known to exhibit carcinogenic and neurotoxic risks (Guenther et al., 2007). In March 2018, a California judge ruled that “coffee firms must add cancer warning on their products”. This verdict was based on the presence of toxic chemicals in coffee, of which only acrylamide was mentioned (CBSLA, 2018; Deabler, 2018; Turner, 2018).

Acrylamide (Figure 1) is an odorless crystalline organic molecule that is primarily used as raw material in the production of polyacrylamide which in turn has various industrial applications. These applications include adhesives, paints and flocculants in addition to the textile and paper industries, wastewater treatment facilities, tunnels and sewers construction, electrophoresis gels and even cosmetics (Shipp et al., 2006). While polyacrylamide is not considered to be significantly toxic (Smith and Oehme, 1991), the acrylamide monomer is known to be toxic to the nervous system and fertility (Miller and Spencer, 1985; Dearfield et al., 1988). Acrylamide exerts genotoxic effects in cells through oxidative DNA damage induced by the build-up of intracellular reactive oxygen species (ROS) and the depletion of glutathione (GSH) (Jiang et al., 2007). In 1994, acrylamide was classified as “a probable human carcinogen, (Group 2A)” by the International Agency for Research on Cancer (IARC) (Anonymous, 1994). In 2002, Tareke et al. first reported the detection of acrylamide in a variety of heat-processed foods such as potato crisps, French fries, bread and

79 hamburgers (Tareke et al., 2002). Few years later, reports claimed that acrylamide is also present in  
80 other food (especially plant derived foods) and beverages, such as biscuits, cereals, wafers, coffee,  
81 almonds, olives, potatoes, sweet potatoes, and baby food (Mizukami et al., 2006; Medeiros Vinci et  
82 al., 2012; Friedman, 2015). Acrylamide is a byproduct in industrially-prepared food/beverages which  
83 is formed as a result of the Maillard reaction of asparagine with reducing sugars, such as glucose,  
84 galactose, and fructose, at high temperatures (Mottram et al., 2002; Robert et al., 2004). The  
85 formation of acrylamide begins at a temperature exceeding 120 °C and reaches its peak at around  
86 170°C (Mottram et al., 2002). One way by which acrylamide levels in food products can be lowered  
87 is by utilizing industrial procedures that convert asparagine into aspartic acid and in the process  
88 lowering the rate of the Maillard reaction (Mottram et al., 2002; Akillioglu, 2014).

89 The World Health Organization (WHO) has set guideline values for acrylamide content in tap  
90 water, yet no country has set any regulations regarding acrylamide content in food and beverages  
91 (Narita and Inouye, 2014). A recent study carried out in our laboratories revealed a high acrylamide  
92 content in local and imported potato-based and corn-based chips (Hariri et al., 2015). In addition to  
93 the high consumption of chips, the Lebanese community has a unique attachment to caffeinated  
94 beverages (Melki, 2016).

95 The present study aims to determine the carcinogenic and neurotoxic risks associated with the  
96 daily consumption of acrylamide from local and imported caffeinated beverages that include  
97 Lebanese coffee, American coffee, espresso and hot chocolate. In line with this target, we aim to  
98 propose to the World Health Organization guideline values for acrylamide levels in caffeinated  
99 beverages.

100

## 101 **2. Materials and Methods**

### 102 **2.1 Acrylamide Analysis**

103 Local and imported brands of ground coffee, coffee beans and hot chocolate were collected  
104 randomly from various locations across Lebanon (Byblos and Beirut) as duplicates with different

production dates. Samples (56) were classified as follows: Lebanese coffee (n = 19), American coffee (n = 5), espresso (n = 5), instant coffee (n = 10) and hot chocolate (n = 17).

### **2.1.1 Chemicals and Reagents**

Chemicals and reagents used in this study were of analytical grade. Acrylamide (standard grade), n-hexane (95%), dichloromethane (DCM, LC-MS grade), propan-1-ol (99%) and acetonitrile (LC-MS grade) were purchased from Sigma–Aldrich, Germany. Distilled water was prepared using a Millipore system.

### **2.1.2 GC-MS Analysis**

Acrylamide was extracted according to the method proposed by Biedermann (Biedermann et al., 2002). A sample of solid hot chocolate or coffee (0.5 g) was mixed with water (5 mL) and heated at 70° C for 30 min. The homogenate was mixed with propanol (10 mL) and centrifuged for 10 min at 2200 rpm and 20° C (for Lebanese coffee, the homogenate was extracted with 2 mL of DCM prior to the addition of propanol). The supernatant was concentrated under reduced pressure. Acetonitrile (2 mL) and hexane (5 mL) were added and the mixture was sonicated for 3 min. The acetonitrile layer was re-extracted with hexane (5 mL), filtered using a C18-SPE cartridge and analyzed on a Gas Chromatogram Mass Spectrometer (GC-MS; Hewlett Packard, HP6890 series) fitted with a fused silica HP5-MS 5% phenyl methyl siloxane cap column; 30.0 m × 250.00 µm i.d., film thickness 0.25 µm; and directly coupled to the MS. The carrier gas was helium with splitless injection and the flow rate of 1.2 mL/min was applied. The temperature program was 15.0 min at 75 °C, from 75 °C to 230 °C at 10 °C/min and hold for 45.0 min. An HP5973 mass selective detector was used in full scan electron ionization (EI) mode. Identification of acrylamide was achieved by comparing mass spectral data against the NIST11 and Wiley9 MS databases along with selective ion monitoring of the molecular ion at an m/z of 71 and a daughter fragment at an m/z of 55. The concentration of acrylamide (µg/kg coffee) was determined by comparing experimental results against a calibration

curve obtained from standard acrylamide solutions prepared in the range of 50-500 mg/L. The limit of quantitation (LOQ) was found to be 5.0 µg/L while the limit of detection (LOD) was found to be 0.5 µg/L. Acrylamide recovery was 92% which is higher than the reported average of 70-80% (Biedermann et al., 2002). The precision and accuracy of the GC method was validated using the Biedermann method by analyzing a 5 g crushed biscuit sample (powder) spiked with 50 mg of standard acrylamide (Biedermann et al., 2002).

## 2.2 Community Survey

A survey targeting the Lebanese population (children, teenagers, young adults and adults) was conducted across south, central and north Lebanon to determine the consumption of hot caffeinated beverages nationwide. Data were collected from 918 subjects from December 2016 to January 2017. Subjects were interviewed about body weight, type of caffeinated beverage, brand, and daily recall consumption. Below is the URL link to the survey conducted:

[https://docs.google.com/forms/d/e/1FAIpQLSc4KSm1rtnGbJD47M5q-P\\_Mq-lf82akrQK-w5BDe7j0nfekA/viewform#start=openform](https://docs.google.com/forms/d/e/1FAIpQLSc4KSm1rtnGbJD47M5q-P_Mq-lf82akrQK-w5BDe7j0nfekA/viewform#start=openform)

## 2.3 Dietary Exposure Assessment

The daily intake of acrylamide consumed from caffeinated beverages by subjects (per body weight) was determined using the following formula:

$$Y = \frac{F \times C_v}{bw}$$

where Y is the daily intake (µg/day), F is the amount of coffee sample (kg) consumed per day, C<sub>v</sub> is the mean acrylamide content in a sample (µg/kg) and bw is the subject body weight (kg). Dietary exposures of a particular age group were reported as a mean, median, maximum, minimum and at the 95<sup>th</sup> percentile.

The margin of exposure (MOE) for neurotoxic risk characterization was determined as the ratio between the NOAEL (no-observed-adverse-effect level) and the dietary exposure of the

157 population (JECFA, 2011). The NOAEL value used was 0.2 mg/kg-bw/day for morphological  
158 changes in rat nerves as determined by the FAO/WHO Expert Committee on Food Additives  
159 (JECFA) (JECFA, 2011).

160 For carcinogenic risk characterization, the MOE was determined as the ratio of the BMDL<sub>10</sub>  
161 (lower limit on the benchmark dose for a 10% response) and the estimated dietary exposure (Michael  
162 Bolger et al., 2010). The BMDL<sub>10</sub> values for acrylamide used in this study were those reported by  
163 JECFA: 0.31 mg/kg-bw/day for the induction of mammary tumors in rats and 0.18 mg/kg-bw/day for  
164 Harderian gland tumors in mice (JECFA, 2011). The mean exposure and the maximum were  
165 calculated and compared with BMDL<sub>10</sub> by calculating the corresponding MOE.

166

## 167 **2.4 Statistical Analysis**

168 Statistical analyses were performed using Microsoft Excel 2016 software (Excel, Redmond,  
169 WA, USA). Statistical significance was considered for  $p < 0.05$ .

170

## 171 **3. Results and Discussion**

### 172 **3.1 Acrylamide Analysis**

173 Duplicate samples with different production dates were extracted and analyzed for their  
174 acrylamide content. For Lebanese coffee, an additional extraction step was carried out using DCM in  
175 order to reduce the interference of the caffeine peak in the GC chromatogram. This was essential as  
176 our preliminary data showed that Lebanese coffee had higher concentrations of caffeine compared to  
177 other caffeinated beverages. GC-MS analysis revealed that the average level of acrylamide in the  
178 tested samples was higher than the limit of quantitation (LOQ; 5.0 µg/L) except for instant coffee  
179 samples. The observed values are reported in Table 1 according to the type of the caffeinated  
180 beverage (Lebanese coffee, American coffee, espresso and hot chocolate) taking into account a  
181 recovery rate of 92%. The variations on the mean might reflect the lack of homogeneity in industrial  
182 sample preparation.

183 Lebanese coffee samples had acrylamide content that ranged from 4,376 to 149,785 µg/kg  
184 with an average value of 50,138 µg/kg and a median of 44,994 µg/kg (Table 1). The average  
185 concentration of acrylamide at the 95% confidence level was found to range between 32,058 µg/kg  
186 and 68,218 µg/kg (t-test). The acrylamide content detected in Lebanese coffee is considerably high  
187 (9- to 19-folds higher) compared to the average value of 3,658 µg/kg of caffeinated product as  
188 reported by Nasreddine and Krishnakumar (Nasreddine et al., 2006a; Krishnakumar and  
189 Visvanathan, 2014) in which various food groups were studied such as caffeinated beverages, meats,  
190 poultry, dairy products, cereals, bread, vegetables, fruits, fats, sugars and alcoholic beverages.

191 Hot chocolate samples had acrylamide content that ranged from 1,168 to 24,120 µg/kg with  
192 an average value of 10,942 µg/kg and a median of 12,931 µg/kg (Table 1). The average  
193 concentration of acrylamide at the 95% confidence level was found to range between 6,743 µg/kg  
194 and 15,141 µg/kg (t-statistic). These values are considerably higher than those reported in the  
195 Chinese market whereby the average acrylamide content in chocolate products ranges between 23 to  
196 537 µg/kg. The samples tested in the Chinese market include dark chocolate, milk chocolate,  
197 chocolate with nuts, chocolate with almonds, and chocolate with wheat best element (Ren et al.,  
198 2006). Our results are in line with those reported by Ren et al. as low-calorie chocolate products  
199 were shown to contain higher acrylamide content compared to regular chocolate products, a result  
200 that may be attributed to the industrial sample preparation (Ren et al., 2006).

201 American coffee had acrylamide content that ranged from 1,074 to 2,900 µg/kg with an  
202 average value of 2,100 µg/kg and a median of 2,144 µg/kg (Table 1). The average concentration of  
203 acrylamide at the 95% confidence level was found to range between 1,210 µg/kg and 2,990 µg/kg (t-  
204 statistic). By relative comparison between the caffeinated beverages in our study, American coffee  
205 contains the lowest range of acrylamide content with considerable variation among brands.

206 Espresso had acrylamide content that ranged from 21,965 to 91,645 µg/kg with an average  
207 value of 38,589 µg/kg and a median of 26,034 µg/kg (Table 1). The average concentration of



acrylamide at the 95% confidence level was found to range between 1,593 and 75,585 µg/kg (t-test), which is higher than the range reported by Taeymans (Taeymans et al., 2004). According to Alves, the level of acrylamide in espresso samples ranges between 131 µg/kg and 2,191 µg/kg (Alves et al., 2010); a range that is significantly lower than the one obtained in this study. In addition, the acrylamide content in espresso samples sold in the Lebanese market ranged significantly from 21,965-91,645 µg/kg and such a huge range can be attributed to the roast degree of different espresso samples (Alves et al., 2010). Hence future work should focus on the determination of acrylamide content across coffee beans subjected to different roasting conditions as well as analyzing and comparing different bean types such as Arabica, Robusta and other blended types.

In the case of instant coffee, acrylamide levels were shown to be below the LOQ. These results are consistent with those reported by Andrzejewski in 2004 (Andrzejewski et al., 2004). This finding can be attributed to the industrial preparation of instant coffee samples whereby the industrial extraction procedure causes the removal of asparagine or converting it into aspartic acid (Mottram et al., 2002; Akillioglu, 2014). Either way, the asparagine concentration is lowered causing the Maillard reaction to slow down and in turn reducing acrylamide concentrations (Akillioglu, 2014).

As previously noted, there are no specific conditions to prepare roast and grind coffee beans and, consequently, variations were observed in the acrylamide levels among duplicate samples. These variations point towards the lack of quality assurance and control by governmental and industrial bodies. However, such a process is essential in the food product industry. Acrylamide content is affected by roasting conditions including variations in temperature, humidity and roasting air velocity (Budryn et al., 2015). In the roasting process, the concentration of acrylamide is at its highest during the middle phase of roasting, and then its degradation proceeds faster than its formation (Lantz et al., 2006). The industrial preparation of caffeinated beverages should therefore have optimized methods which are under continuous governmental control in order to ensure low acrylamide levels and consumer safety. In addition, decaffeinated samples were found to contain up

233 to 3-fold more acrylamide compared with caffeinated samples of the same brand. This variation may  
234 be attributed to the decaffeination process that entails five steps (Chu et al., 2013). In the  
235 decaffeination process, the swelling of beans is carried out in water after which caffeine is extracted.  
236 Stream stripping is applied to remove all solvent residues from the beans. Finally, the adsorbents are  
237 regenerated and the decaffeinated coffee beans dried to their initial moisture content (Chu et al.,  
238 2013). In short, the decaffeination process subjects the coffee beans to higher temperatures for longer  
239 periods of time. As a result, the acrylamide content in decaffeinated products is enhanced, rendering  
240 the decaffeinated alternative a high source of the carcinogenic and neurotoxic acrylamide. The  
241 observed high acrylamide concentrations in the various groups of caffeinated beverages should  
242 encourage the development of novel approaches in order to reduce the acrylamide content in certain  
243 foods such as coffee and its derivatives.

244 Recently, Budryn et al. investigated the influence of both velocity and humidity of roasting  
245 air at different temperatures on the concentration of acrylamide in roasted beans (Budryn et al.,  
246 2015). The study revealed that increasing the air velocity, regardless of the temperature, resulted in  
247 the intensification of acrylamide formation. Furthermore, increasing the roasting air humidity caused  
248 a lowering of acrylamide formation at high roasting temperatures. This was accompanied with an  
249 increased degradation of polyphenols and the deterioration of their sensory characteristics and  
250 antioxidant activity. The optimum temperature for moderate degradation of polyphenols was  
251 reported to be 203 °C. However, the correlation between high temperature and high acrylamide  
252 levels cannot be ignored. A recent study on roasted green coffee beans which were incubated in  
253 water at a maximum temperature of 90 °C noted an 80% reduction in acrylamide content compared  
254 to non-incubated beans (Navarini et al., 2014). The end products of this procedure were dry  
255 reincorporated green coffee beans that are low in asparagine and aspartic acid. These findings  
256 indicate that controlling the roasting procedure (temperature and pressure) affects the acrylamide  
257 content. During a visit to one of the local coffee chains, we observed that the same coffee beans were

used in the preparation of both American coffee and espresso; however, the difference in acrylamide content based on our GC-MS analyses could not be ignored. While examining the machinery used in the sample preparation, it was noticed that American coffee was prepared using machinery equipped with a cooling system while the machinery used for espresso samples subjected the beans to higher pressures and temperatures.

### **3.2 Dietary Intake of Acrylamide from Coffee**

Evaluation of the dietary exposure to acrylamide from caffeinated beverages was calculated by combining the average concentration per kg sample and the consumption data from the survey we conducted. In the present study, the deterministic model was used as a preliminary approach to assess the dietary exposure of acrylamide from caffeinated beverages (Dybing et al., 2005). The daily consumption of acrylamide from the various caffeinated beverages (barring instant coffee) was found to be 10.9 µg/kg-bw/day (Lebanese coffee), 7.4 µg/kg-bw/day (Espresso), 1.2 µg/kg-bw/day (hot chocolate) and 0.37 µg/kg-bw/day (American coffee) with an average of 5.02 µg/kg-bw/day combining all beverages. The highest intake rate was observed among adults (41-50 years old) who consume Lebanese coffee at a rate of 12.5 µg/kg-bw/day.

In an attempt to estimate the average daily intake of acrylamide from various food products among the Lebanese population, Nasreddine's results were updated/modified (Table 2) based on a recent study carried out in 2014 by Krishnakumar (Nasreddine et al., 2006b; Krishnakumar and Visvanathan, 2014). The updated data show that caffeinated beverages account to 46.9% of the total dietary intake of acrylamide. As such, the average daily intake of acrylamide from various food products in the Lebanese population is estimated to be 10.7 µg/kg-bw/day. This value is much higher than that reported by the WHO which estimates the total average of acrylamide dietary intake to vary between 0.3 and 2 µg/kg-bw/day (FAO/WHO, 2002; WHO/JECFA, 2005). The results for the Lebanese population are also higher than the maximum risk intake of 4 µg/kg-bw/day set by the WHO in 2005 (WHO/JECFA, 2005). Alarming, the mean acrylamide intake, from caffeinated

284 beverages alone (never mind the total acrylamide dietary intake), among the Lebanese population is  
285 still higher than the risk intake set by the WHO.

286 For instance, subjects with high consumption of Lebanese coffee are subject to an acrylamide  
287 intake of 20.6  $\mu\text{g/kg-bw/day}$ . This value is 5-fold higher than the maximum risk intake of 4  $\mu\text{g/kg-}$   
288  $\text{bw/day}$  set by the WHO (WHO, 2005). Accordingly, quality and quantity of caffeinated beverages  
289 sold in the Lebanese market should be regulated. The high acrylamide levels found in this study  
290 further support the call by the WHO, EFSA (EFSA, 2011), Japan, Hong Kong, EU, Canada and  
291 International Organizations to set governmental control in order to limit acrylamide levels in food  
292 products.

293

### 294 **3.3 Risk evaluation of Acrylamide Consumption**

#### 295 **3.3.1 Neurotoxicity**

296 The mean exposure of the Lebanese population to acrylamide from caffeinated beverages was  
297 determined as 5.02  $\mu\text{g/kg-bw/day}$  and is 25-fold higher than the reference does (RfD) of 0.2  $\mu\text{g/kg-}$   
298  $\text{bw/day}$  set by the US-EPA based on neurotoxic investigations in rats (US-EPA, 1993). In 2011,  
299 JECFA set the minimum margin of exposure (MOE) for acrylamide as 200 for the mean dietary  
300 exposure and 50 for the high dietary exposure (JECFA, 2011). Among the population of consumers  
301 of caffeinated beverages, the acrylamide MOEs ranged from 18 to 535, depending on the choice of  
302 beverage (Figure 3). The average MOE values (presented as Population MOE; MOE range for all  
303 age groups) of acrylamide from Lebanese coffee (18; 16-24), espresso (27; 21-33) and hot chocolate  
304 (163; 144-182), among all age groups, were all well below the set value of 200 and thus a significant  
305 neurotoxic risk can be predicted for all consumers (Table 3). While it is expected that people above  
306 the age of 50 would display a higher neurotoxic risk as a result of high coffee consumption, the  
307 survey results also show that the elder generation consume more hot chocolate compared to younger  
308 generations, and hence, is subjected to an even higher neurotoxic risk. As for American coffee, the  
309 average MOE values of acrylamide (441-775) among the Lebanese population were found to be

above 200 and thus unlikely to have adverse neurological effects at the estimated average exposure; however, morphological changes in nerves cannot be excluded among high consumers of American coffee as reported (JECFA, 2011). To the best of our knowledge, this is the first study to report neurotoxicity as a result of acrylamide dietary intake which may indicate that acrylamide levels in food products are on the rise and hence tighter and stricter legislations are urgently needed to ensure consumer safety. As for the mean total acrylamide dietary intake, the MOE for neurotoxicity alarmingly extrapolates to a low of 19 which is 11-fold more neurotoxic than the set limit of 200.

317

### 3.3.2 Carcinogenicity

The average exposure to acrylamide from caffeinated beverages was found to be 63 times higher than the intake of 0.08  $\mu\text{g/kg-bw/day}$ , as estimated by the NFCA (NFCA, 2002) and 36 times higher than the intake of 0.14  $\mu\text{g/kg-bw/day}$  as set by the WHO (WHO, 1996). According to JECFA, a genotoxic and carcinogenic compound like acrylamide may raise concern if low MOEs (below 310 or 180 depending on the  $\text{BMDL}_{10}$  value considered) are observed (JECFA, 2011). The MOEs for carcinogenicity as calculated for the entire population mean exposure to acrylamide from various caffeinated beverages ranged from 28-829 at  $\text{BMDL}_{10}$  (0.31  $\text{mg/kg-bw/day}$ ) and 17-481 at  $\text{BMDL}_{10}$  (0.18  $\text{mg/kg-bw/day}$ ). Results are presented in the following format  $\text{BMDL}_{10}$  0.31 ( $\text{BMDL}_{10}$  0.18). The acrylamide MOEs of the entire population from Lebanese coffee 28(17) and espresso 42(24) were alarmingly low at both  $\text{BMDL}_{10}$  values (Table 3), indicating a highly carcinogenic risk for the consumers. The acrylamide MOEs of the entire population from chocolate products 252(139) are close to the limit values and hence only high consumers could exhibit carcinogenic risk. Finally, American coffee once again seems to exhibit a lesser carcinogenic risk for all ages as acrylamide MOEs of 829(481) were higher than the WHO set values. As for the mean total acrylamide dietary intake, the MOE for carcinogenicity extrapolates to 29 ( $\text{BMDL}_{10}$  310) and 17 ( $\text{BMDL}_{10}$  180) which is approximately 11-fold more carcinogenic than the set limit.

Based on the average body weight and the  $\mu\text{g}$  content of acrylamide/kg sample, we have

336 calculated the maximum amount of cups (1 cup correlates with 5 g of coffee beans/powder), for each  
337 caffeinated beverage, that should be consumed by the Lebanese population per day in order to avoid  
338 a carcinogenic risk at the BMDL<sub>10</sub> 300 level. The results can be summarized as follows: Lebanese  
339 coffee (1.44 g of product, 0.3 cups), espresso (1.89 g of product, 0.4 cups), hot chocolate (6.4 g, 1.3  
340 cups) and American coffee (33.2 g, 6.6 cups). Only consumers of American coffee (2.4 cups/day)  
341 seem to consume less than the tolerable daily intake; however, consumption of Lebanese coffee and  
342 espresso is high and it might be unlikely that these consumers could easily cut down their  
343 consumption from 3.0 and 2.7 cups to as low as 0.3 and 0.4 cups, respectively.

344 In an attempt to set the limit of acrylamide in caffeinated beverages whereby no neurotoxicity  
345 (MOE above 200) or carcinogenicity (MOE above 310) would be observed among consumers, we  
346 combined the consumption data from the survey along with acrylamide concentration/sample while  
347 also taking the average body weight into consideration. The concentration of acrylamide that would  
348 generate a carcinogenic MOE value of 310 comes out to 14,400 (Lebanese coffee), 14,620  
349 (espresso), 13,940 (hot chocolate) and 13,940 (American coffee) µg acrylamide/kg sample (MOE  
350 310 was considered as the risk of carcinogenicity is higher than the risk of neurotoxicity). In order to  
351 avoid carcinogenic and neurotoxic risks, we propose that WHO/FAO set the average acrylamide  
352 levels in caffeinated beverages to 7,000 µg acrylamide/kg sample, a value which sets at  
353 approximately 50% of the limit acrylamide concentration for carcinogenic risk considering that  
354 caffeinated beverages account for almost 50% of the total acrylamide dietary intake. The proposed  
355 concentration is 4-fold lower than the investigated average acrylamide level of 29,176 µg/kg sample  
356 in caffeinated beverages sold in the Lebanese market.

357

#### 358 **4. Conclusion**

359 The results of this study indicate that the acrylamide levels in caffeinated beverages sold in  
360 the Lebanese market are significantly high and further supports that the ruling of the Californian  
361 court to add a cancer warning on coffee products is valid (CBSLA, 2018; Deabler, 2018; Turner,

2018). Lebanese coffee, espresso and hot chocolate were shown to induce significant carcinogenic and neurotoxic risks at both the mean and high dietary exposures; while American coffee was shown to pose a risk mainly for consumers with high dietary exposure. Based on these results, the need for strict and scientific legislations to control the coffee industry and protect the consumer is a must. We propose that the WHO and FAO set optimal procedures for coffee extraction and preparation including roasting times and pressure/etc. It is also crucial that coffee batches get tested for their acrylamide content before being sent to the market. Based on our results, we propose that the WHO/FAO set the acrylamide levels in caffeinated products to 7,000 µg/kg product. Last note, we are further investigating all known acrylamide containing food products in the Lebanese market in order to extrapolate an updated total acrylamide dietary intake.

#### **4. Acknowledgment**

We thank the School of Arts and Sciences and School of Pharmacy at the Lebanese American University for providing the funding and facilities to carry out the analysis described in this manuscript. We thank the National Council for Scientific Research, CNRS Lebanon (CNRS/02-01-18), for providing the funding for research assistants and chemicals.

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