

# Assessment of cadmium levels in chocolate



## Research Proposal

### Assessment of Cadmium levels in chocolate commercialized in Lebanon

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## **1- Background and significance**

Cadmium (Cd) as an element is a soft silver-white transition metal. It is not usually present in the environment as a pure metal, but is most often present in the form of oxides, sulfides, and carbonates. It does not have a taste or odor. Cadmium sulfate and cadmium chloride are quite soluble in water, whereas metal Cd, cadmium oxide and cadmium sulfide are almost insoluble (International Program on Chemical safety [IPCS], 2007).

Cd is released from several sources in nature: mobilization of Cd from the Earth's crust and mantle due to volcanic activity, mobilization of Cd impurities in extracted raw materials such as phosphate minerals and fossil fuels, release of Cd from products and processes resulting from the use, disposal, recycling, open burning or incineration, releases from municipal installations and release of Cd previously deposited in soils, sediments, landfills and waste or tailings piles (United Nations Environment Program [UNEP], 2010).

It is a toxic metal to humans and classified as a carcinogen by the National Toxicology Program (Agency for Toxic Substances and Disease Registry [ATSDR], 2004). In the human body, Cd is mainly stored in the liver and kidneys. Hence, an early effect indicator of Cd toxicity is increased excretion of proteins in urine, kidney proteinuria, which is the result of proximal

tubular cell damage. Several other side effects occur depending on the duration and magnitude of exposure.

Skeletal damage is another critical effect of chronic Cd exposure at high levels. Cd concentrations in most tissues increase with age since excretion is normally slow, and the biological half-life is very long (10 to 15 years) (Jin T., Lu J. & Nordberg M., 1998) in the muscles, kidneys, liver and whole body. In exposed people with renal damage, urinary excretion of Cd increases and so the whole body half-life is shortened. The kidney burden resulting from cumulative exposure to Cd can be assessed by measuring Cd in urine (UNEP, 2010).

Epidemiological and experimental studies have associated occupational Cd exposure with several types of cancers including lung, prostate, renal, liver, hematopoietic system, urinary bladder, pancreatic, testis and stomach cancers (Journal of Inorganic biochemistry, 2000; Joseph P. et al., 2001). Exposure to this toxic metal also severely affects the function of the nervous system (L'opez E. et al., 2003; Cao Y. et al., 2009), with symptoms including headache and vertigo, olfactory dysfunction, Parkinson-like symptoms, slowing of vasomotor functioning, peripheral neuropathy, decreased equilibrium, decreased ability to concentrate and learning disabilities (Cao Y. et al., 2009; Phil R. O. & Parkes M., 1977). Presence of Cd was also detected in hair and higher concentrations of hair Cd were reported in children with mental retardation (Marlowe M., Errera J. & Jacobs J., 1983) and learning difficulties or dyslexia (Phil R. O. & Parkes M., 1977; Capel I. et al., 1981).

There are several sources of human exposure to Cd including employment in metal industries, production of certain batteries, some electroplating processes and consumption of tobacco products (International Agency for Research on Cancer [IARC], 1993). However, food accounts for approximately 90% of the Cd intake in the general, non-smoking population since this metal is found in the soil. The quantity absorbed by crops in different locations is influenced by factors such as soil pH, salinity, crop species and varieties and the presence of other elements (e. g., zinc). Less than 10% of the total exposure occurs due to inhalation of Cd in ambient air or ingestion with drinking water (UNEP, 2010).

Since Cd is dangerous to humans, a provisional tolerable weekly intake (PTWI) or provisional tolerable monthly intake (PTMI) had to be established. PTWI is an estimate of the amount of the chemical that can be ingested weekly over a lifetime without appreciable health risk (Food and Agriculture Organization [FAO] & World Health Organization [WHO], 1988). The European Union recommends a PTWI of 2.5  $\mu\text{g}/\text{kg}$  of body weight (European Union [EU], 2014). The PTMI for Cd recommended by the FAO/WHO Expert Committee on Food Additives (ECFA) is 25  $\mu\text{g}/\text{kg}$  of body weight (FAO & WHO, 2014). In 2010, the Consumer Product Safety Commission (CPSC) recommended that the acceptable daily intake level of 0.1  $\mu\text{g}/\text{kg}$  body weight per day for chronic exposure (Mead N., 2010).

Studies in several European countries have demonstrated high levels of Cd in agricultural topsoil due to the use of Cd in fertilizers and atmospheric deposition. Over the last 100 years, the increase in soil Cd concentration in Austria, Denmark, Finland, Greece, Ireland and the United Kingdom was

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estimated to be 7 to 43 percent (UNEP, 2010). Hence, the risk is in continuous increase and further investigation on the quality of the crops and food consumed has to be pursued.

Out of the possible crops carrying Cd, cocoa, the seed of the *Theobroma cacao* tree (Watson R., Preedy V. & Zibadi S., 2013; Lee F., 1983), is one of the most consumed by all age groups worldwide, especially by children. The large consumption of cocoa and chocolate products derived from cocoa is due to its pleasant flavor and the feeling of well-being that it gives (Watson R., Preedy V. & Zibadi S., 2013). Several studies revealed the benefits of chocolate consumption due to the high levels of flavonoids and antioxidants present in cocoa based foods (Grivetti L. & Shapiro H., 2009; Crozier S. al., 2011; Buitrago-Lopez A. et al., 2011). They are an important source of minerals such as Ca, P, Fe, Mg, Cu, Zn, K, and Mn (Grivetti L. & Shapiro H., 2009; Peixoto R., Oliveira E. & Cadore S., 2012; Pedro N., Oliveira E. & Cadore S., 2006). In addition, they may prevent harmful effects caused by free radicals in the human body, contributing to the reduction of cardiovascular disease and cancer risk (Fernandez-Murga L. et al., 2011; Yao H., 2011). However, the presence of potentially toxic elements has also been reported (Rehman S. & Husnain M., 2013; Yanus R. et al., 2014), particularly lead and Cd (Dahiya S. et al., 2005; Jalbani N. et al., 2009).

Previous studies have been performed to test the presence of Cd in chocolate samples in several countries and the results revealed the presence of this heavy metal with a large margin of variation. In turkey 20-30 ppb were observed, in India 1 to 2730 ppb were reported, in Malaysia 280 to 420 ppb and in Pakistan 4. 3 to 190 ppb were observed (Dahiya S. et al., 2005;

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da Silva A. et al., 2006; Dos Santos A. et al., 2005; Guldás M. et al., 2008; Lee P. and Low T., 1985; Leggli C. et al., 2011). In Oakland, California, the non-profit organization As You Sow (AYS) tested Cd levels in 42 products, 26 of which contained lead and/or Cd level above what the state of California considers safe. The organization sent legal notices in the context of holding more heavy metals than allowed under the Golden State's Proposition 65 toxic chemical warning law to 16 manufacturers including Hershey's, See's, Mars, Godiva, Ghirardelli, Lindt, Green and Black's, Kroger, Whole Foods, Trader Joe's, Earth Circle Organics, Moonstruck, Theo, and Vosges (AYS, 2015; The Washington Post, 2015).

In this study, given that some of the brands commercialized worldwide are found in Lebanon, cocoa and chocolate derivatives will be tested for their Cd content.

### 1. Specific Aim

Various brands of chocolate, whether local or imported brands, are largely consumed in Lebanon. The main ingredients in chocolate consist of cocoa, milk and fats, each of which is a potential source of Cd. No study has been conducted till now to assess the quantity of toxic metals present in the products on the market. Hence, a study to determine Cd levels is important for chocolate consumers and manufacturers.

The aim of this study is to assess Cd levels in chocolate samples mostly consumed by the Lebanese population and compare those levels to the values issued by global health organizations.

### 2. Research design and methods

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## 1. Sample collection

Different brands of dark chocolate, milk chocolate and cocoa powder are collected from different stores according to the most sold brand. The shelf life of most milk chocolate samples is one year, and 2 years for dark chocolate. Samples will be labeled and stored at a temperature between 15 and 17 °C. Samples will be kept wrapped in foil and placed inside a Ziploc plastic bag so that they do not absorb the odors and moisture from the refrigerator (Subarmanian P., 1998).

## 2. Microwave digestion

In order to detect heavy metals in cocoa, the samples have to be digested since the matrices are organic. According to the Environmental Protection Agency (EPA), microwave digestion can be followed on chocolate samples where up to 0.5g are digested in 5mL of concentrated nitric acid and up to 2mL hydrogen peroxide (Onianwa P. C. et al. 1999; Mounicou S. et al., 2002), which can also be substituted by nitric acid and hydrofluoric acid (Güldağ M., Adnan F. D., & Biricik F. B., 2008). The digestion is run in PTFE vessels at approximately  $180 \pm 5$  °C for 15 minutes (Environmental Protection Agency [EPA], 2004). The time and temperature are subject to variation in order to find the most convenient parameters for the samples tested.

Wet digestion method can also be applied in the digestion of chocolate samples. The two methods are comparable in results (Jalbani N. et al., 2009). However, wet digestion requires a greater use of chemicals and causes a greater risk of contamination (Jalbani N. et al., 2009).

As the samples will be used to assess both lead (Pb) and Cd, each sample is spiked with an internal standard of Pb and Cd prior to digestion (FDA, 2014). Obtained samples after digestion are reconstituted to 25mL with ultrapure distilled water in volumetric flasks then placed in vials and stored in the refrigerator (EPA, 2004; Jalbani N. et al., 2009).

### 3. Atomic absorption analysis

For quantitation, stock Cd and lead standard solutions of 1000mg/L each are diluted to different concentrations and a standard addition method is followed to prepare a calibration curve. Diluted Cd and lead stock standards with 1% nitric acid will be placed in nitric acid rinsed volumetric flask and stored in plastic bottles (Teflon<sup>®</sup> FEP or HDPE bottles recommended). Both elements can be combined in the same solution (Food and Drug Administration [FDA], 2010).

The heavy metal is detected by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). Electrodeless discharge lamps for Cd and Pb are operated, alternatively. Cd will be detected at a wavelength of 228.8 nm (FDA, 2010). Pure argon (99.999%) is used as the purge and protective gas. Different chemical modifiers will be tried to find the most convenient one such as ammonium phosphate, magnesium nitrate, phosphoric acid or others.

### 4. Statistical analysis

Statistical analysis will be done in two different stages. First, the mean Cd concentration of each brand of chocolate will be calculated and proportion differences for independent samples will be tested by comparing the

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calculated mean values to the permitted ones by Global Health Organizations.

If significant differences are observed, a second step of analysis will be performed in which the studied brands are subcategorized forming a contingency table. The differences can be identified by a  $\chi^2$  test thus allowing the formation of an ANOVA study to check in depth for the differences within the categories and between them. Using these methods, the most diverging categories from the norm will be identified.

### 3. Expected results

This study will most likely reveal the presence of Cd in chocolate samples as it has been the case in other similar studies. Some samples might have values higher than the tolerable intake specified by global health organizations.

### 4. Potential problems

Cd is toxic at low doses (FAO & WHO, 1988; Mead N., 2010; EU, 2014; FAO & WHO, 2014) and may be found in low doses in some samples as shown in previous studies (Dahiya S. et al., 2005; da Silva A. et al., 2006; Dos Santos A. et al., 2005; Guldaz M. et al., 2008; Lee P. and Low T., 1985; Leggli C. et al., 2011). Accordingly, the selection of an appropriate chemical modifier for AAS analysis is very important. Several trials will be attempted in order to select the optimal type. In addition, the order and receipt of chemicals will be time consuming.

### 5. Time frame

Completion of the proposed aim requires duration of 3 to 4 months in order to allow for the collection of samples, sample digestion, graphite furnace analysis, evaluation and statistical analysis of the results obtained.