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RESEARCH PAPER

Determination of Dioxins and Polychlorinated Biphenyls Levels in Milk Samples from Capital and North of Iran

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ABSTRACT

High levels of dioxins and dioxin-like compounds in the food and their adverse effects on human health are of increasing concern. Since milk is one of the most essential human nutritional resources, the present study aims at determining dioxins and PCBs in raw milk samples from four farms in North of Iran and raw and pasteurized samples from three farms and five dairy factories in Vicinity of Tehran (capital of Iran). Total toxic equivalence (TEQ) of dioxin and PCBs have been determined, using the DR-CALUX® bioassay. Results reveal that all samples are contaminated with dioxins and PCBs, comparatively. The total dioxins and PCBs levels in raw milk samples from the north range from 4.08 to 0.97 pg/gfat and for the raw samples and pasteurized samples from Tehran Province from 1.89 to 0.63 pg/gfat and 0.1 to 0.03 pg/gfat, respectively. The mean concentration of dioxins/PCBs is higher in samples from the north of Iran. This may be because of the common method of removing domestic and agricultural disposal in this area.

Keywords: Environmental pollution, Persistent Pollution, Dairy, food sample, DR-CALUX

INTRODUCTION

Dioxins and dioxin-like compounds (polychlorinated biphenyls (PCBs)) are the environmental pollutions that release to the atmosphere as unwanted by-products of many chemical processes i.e. wildfire, incineration, combustion, and industrial processes (Malisch, 2017) . Dioxins are aromatic hydrocarbons, which can be halogenated in various degrees. The term dioxin refers to a class of family that includes polychlorinated dibenzo-ρ- dioxin (PCDDs), polychlorinated dibenzofuran (PCDFs) and non-ortho and mono-ortho polychlorinated biphenyl (PCB) (Cole et al., 2003). Due to their resistance to physicochemical and biological degradation, they are persistent pollutants for the environment and have the ability to transfer from the source of emission and bioaccumulate. They are found in air, water, sediment and transfer to the food chains (Kanan and Samara, 2018) . Dioxins cause harmful effects even in low doses and can induce immune and reproductive toxicity, along with enzyme induction, developmental effects, and carcinogenesis (Srogi, 2008; Tuomisto, 2019).

The most important source of human exposure is contaminated food consumption (Weber et al., s2018). Dioxins and PCBs are found in almost all foodstuff but owing to their hydrophobic nature, the highest concentration observes in animal origin food like fish, meat, and dairy products (De Mul et al., 2008; Fernandes and Falandysz, 2021).

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Due to persistence and severe toxicity, the level of dioxins in foodstuff is a health issue. To prevent human exposure to dioxins, WHO (World Health Organization) and European Commission established recommended tolerable daily intake of 2 pg TEQ/ kg per day (European Commission (EC), 2001).

Milk and dairy products are parts of a healthy diet. Based on previous studies on human dietary uptake, milk and dairy products have known as a major contribution of dioxins and PCBs (Malisch et al., 2000). According to previous researches in Iran, the concentration of dioxins in milk samples and dairy products was high and health-threatening (Rezaei et al., 2015; Ahmadkhaniha et al., 2017).

For detecting dioxins and dioxin-like compound various method has been used, and high-resolution gas chromatography/mass spectrometry (HRGCM) has been called “golden standard” for dioxins detection, but applying this method on all samples is very time-consuming and expensive (Sany et al., 2016).

Amongst several approaches, the method offering the most cost-effective alternative is the cell-based screening method of the Dioxin-Responsive Chemical-Activated LUciferase gene eXpression (DR_CALUX) assay (Scippo et al., 2004; van Vugt-Lussenburg et al., 2013). In this project, for the first time in Iran, DR-CALUX ® set up technique was selected.

To our knowledge, data about dioxins levels in milk lacks in the northern and central Iran samples. According to the geographical and industrial conditions in these regions, there are many concerns about the presence of pollution in the environment and food chain. Hence, this study was designed to monitor cow milk samples from these regions.

MATERIAL & METHODS

The stable cell-line and eight TCDD standard solutions supplied by BioDetection Systems (BDS, Amsterdam, The Netherlands). Chemicals were provided from various suppliers; Silica 60 (63-200µm), sulfuric acid, n-hexane, Isopropanol, and Diethyl ether from Merck (Germany), Dimethyl sulfoxide (DMSO)and anhydrous sodium sulfate from Merck (Germany), D-luciferin from Resem (Netherland), and α-MEM and Gibco from Invitrogen (Germany).

Thirty-two samples were collected totally. Twelve raw milk samples were collected from four different farms in the northern Iran during three stages from November 2015 to Jun 2016. Nine raw milk samples were collected from 3 different farms in Tehran province. Also, 15 pasteurized milk samples produced by five different companies in Tehran province were collected in three stages over the 9-month study period. Samples were stored at 4º C until extraction.

The total toxic equivalence (TEQ) of dioxins and PCBs were determined by the DR-CALUX bioassay, which developed by Wageningen University in 1993. and distributed by BioDetection System (BDS, NL). It is a validated approach for screening dioxins and PCBs in feed and food according to international standards, like EC/252/2012 and EC/278/2012 guideline (Arkenbout and Esbensen, 2017). DR-CALUX bioassay is developed based on the affinity of dioxins and PCBs to bind to Aryl-hydrocarbons (Ah) receptors. This bioassay recruits genetically modified rat heptoma H4IIE cell lines that express the firefly Luciferase (gene reporter) under control of Ah receptors, in present of dioxins and PCBs (Murk et al., 1997; Budin et al., 2020).

For each sample, all stages of the protocol were performed as described by BDS. In brief, milk lipids extracted from a certain amount of milk samples by n-hexane and Isopropanol extraction solutions. In the extraction step, dioxins/furans and PCBs were not separated. Then, extracted material was cleaned up via sulphuric acid-silica column and n-hexane/diethyl ether (97:3, v/v). The fraction contains dioxins and dioxin-like compounds. After elution, the solvent evaporated, then DMSO was added for storage. To performed DR-CALUX® analysis, cells were exposed to the mixture of samples extract and cell media for 24 hours (in triplicate, in 96 wells plates). After incubation, cells were lysed and Luciferase substrate added (Luciferin, ATP, etc.). Then, the activity of Luciferase was assessed by Luminometer (Berthold Detection Systems, Germany). Finally, luciferase activity was compared with the standard curve of the most toxic congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), to calculate the TEQ value (expressed as pg TEQg_1 fat).

Although a great correlations have been established between DR-CALUX-TEQs and high-resolution gas chromatography- high-resolution mass spectrometry (HRGC_HRMS) TEQs for PCBs or PCBs with dioxins/furans mixtures (Joung et al., 2007), to test the efficiency and accuracy of our setup, additional analysis by HRGC_HRMS was conducted on a raw milk sample from northern Iran. The sample was sent to Nofa Lab, Netherland for chemical investigation. It should be noted that for statistical analysis R program software was used (R i386 3.6.1).

RESULTS & DISCUSSION:

Total dioxins and PCBs levels are shown in Table1. Results showed that dioxins/PCBs were detectable in all samples in varying extent. The highest TEQ level was belonged to the raw milk samples from the northern Iran ranged 4.08-0.97 pg/gfat with the mean of 2.42 ± 0.97.

Table1. Total dioxin and PCB levels in milk samples (pgTEQg_1 fat)

	Number of samples	Mean ± SD	Range
Raw samples from Northern Iran	12	2.42 ± 0.97	0.97-4.08
Raw samples from Central Iran	9	1.13 ± 0.4	0.63-1.89
Pasteurized samples from Tehran	15	0.06 ± 0.02	0.03-0.1

In samples from three farms in the vicinity of Tehran, the levels ranged from 0.63 to 1.89 pg/gfat, with mean of 1.13 ± 0.4 pg/gfat. Pasteurized samples showed the lowest dioxins levels in all groups (mean of 0.06 ± 0.02 pg/gfat).

Furthermore, a good agreement was found between DR-CALUX® and GC–HRMS regarding dioxins and PCBs concentration (HRGC/HRMC: 2.8 pg/gfat vs. DR-CALUX®: 3.01). Noticeably, a relatively higher measure by DR-CALUX® might be explained by the fact that a variety of ligands poses affinity to bind to AhR, for example brominated compounds that are co-extracted with dioxins and chlorinated compounds (i.e. PCBs). Comparing to WHO recommended tolerable limit (2 pg TEQ/g per day), dioxin’s level in the samples from north of Iran is considerably high and has a negative impact on consumer’s health. Therefore, pre- treatment to remove fat-soluble dioxin before consumption is mandatory.

Given the importance of this issue, high distribution, persistence, and severe toxicity of dioxins, and with the expanding industrial regions, several international conventions were established to monitor the concentration of dioxins in sediments and foodstuff. The result of strict legislation and control can be seen in the comprehensive study that showed dioxins emission during 1990-2017 revealed a relative declining trend in most countries in European Union, Canada, and Australia (Salian et al., 2019). In 2008, Durand *et al.* determined the concentration of dioxins in cow's milk samples collected from 17 companies in different regions in France. The average concentration of dioxins was 0.33 pg TEQ/g which was cut by half in comparison to a previous survey conducted in 1998 analyzing milk samples from France. In this study, the concentration of dioxins in all samples did not exceed the limit set by European Commission and was relatively lower than the results of our study(Durand et al., s2008). In 2021, Cavallo *et al.* studied the occurrence of PCCD/Fs and PCBs in milk samples taken from farms located in the Campania region, Italy, in the period of 2008 to 2018. The average concentration of dioxins and PCBs was 0.54 pg TEQ/g which was well below the EU regulatory limit and this report showed a decreasing trend of dioxins contamination over 10 years in this region (Cavallo et al., 2021).

Unfortunately, only limited studies have been conducted on this issue in the Middle East, and one of the most challenging problems in controlling and management of dioxins in the Middle East countries is the lack of information and data on contamination of dioxins in soil and water. Due to the industrialization of countries in this region and the increase in dioxins emissions, further studies are vital for protecting the environment and human health (Hajjar, 2012; Kanan and Samara, 2018). In 2016, a study was conducted on the level of dioxins in certain Egyptian cow's milk. The results showed that the level of dioxins in milk samples with 3.9 pg TEQ/g was higher than the acceptable European standard limits. According to this study, the general exposure of the population in Egypt to the dioxins was through cow milk and other animal-origin food (El-Nawawy et al., 2016).

As well, there is not comprehensive information and data on the level of dioxin's contamination in soil and sediment, water grounds, and foodstuff in Iran. In 2015, Ahmadkhaniha and *et al.* determined the concentration of dioxins-like (PCBs) in commercial pasteurized milk in Tehran at two different seasons. The sum of PCB residues was 0.42 pg TEQ/g which considerably lower than the defined threshold set by the European Commission, and the seasonal variations were not observed in this study (Ahmadkhaniha et al., 2017). In this project, they just analyzed the concentration of PCBs in milk samples, while using DR-CALUX, allowed us to detect and quantify all dioxins (PCCD/Fs) and dioxin-like congers (PCBs) members.

In 2015, Rezaei et al. determined the concentration of PCDDs in 15 samples of pasteurized milk from three main dairy companies of Khuzestan province (South of Iran). The result showed that dioxins were found in all samples comparatively in the range of 0.59-3.17 pg TEQ/g that is far more the result of our project for pasteurized milk samples in Tehran province (0.03-0.1) (Rezaei et al., 2015). We did not record the effect of the seasons in our study. In contrast, Rezaei found that the concentration of dioxin in pasteurized milk samples were season dependent.

There is no information on dioxin's concentration in foodstuff including milk and dairy products in other regions in Iran. The main producer of dioxins in Iran is the industrialized provinces like Yazd, Semnan, Khorasan, Qazvin, and Qom. The concentration of dioxins in the north part of Iran is considerable too. Iran seems to be a significant producer of these compounds in comparison with fifteen industrialized countries (Azari et al., 2007).

In our study, the results showed that dioxins concentration in pasteurized milk samples collected from five factories in Tehran was well below the limit of the European Commission (mean: 0.06 pg TEQ/g). The mean level of dioxins in raw milk samples collected from three farms in the vicinity of Tehran was 1.13 pg TEQ/g, that is near the defined threshold. The third group of samples, the raw milk samples collected from farms in north of the Iran (Gilan), had the highest concentration of dioxins and exceed the limit set by WHO and European Commission. This result can be explained by the main disposal method in this region; open burning of agricultural residues, and its role in dioxins emission. According to the study on the sources of emission of dioxins in Iran, improper and uncontrolled waste burning might account for the major source of dioxins emission in the north and northeast provinces of Iran (Momeniha et al., 2011). Similarly, studies in China and India showed that the open burning of domestic and agricultural wastes shares a high proportion of dioxins and dioxin-like compounds emission into the environment (Zhang et al., 2008; Kumari et al., 2019). Therefore, these results were predictable in the samples from the north of Iran.

The high incidence of cancer in the northern provinces of Iran has caused many concerns(Beiranvand et al., 2018). Numerous studies have been conducted in this area to evaluate the level of various pollution including agricultural chemicals, pesticide and heavy metals in soil, water and foodstuff (Faraji et al., 2014; Ebadi and Hissoriev, 2017; Ghanbari et al. , 2020). Our study provided useful information about dioxins contamination in this region.

In this project, the DR-CALUX technique was used for the first time in Iran to detect and quantify the concentration of dioxins in milk samples. This is a trustable and validated method for screening dioxins in large samples and a cost- and time-saving alternative for the conventional method that has been widely used in recent studies all over the world.

CONCLUSION

Considering Iran as a developing country that is moving toward civilization and industrialization rapidly, population health maintenance is an upcoming challenge. Hence, regular monitoring of dioxins in food products in reference laboratories is an important practice. Moreover, controlling dioxins/PCBs emission into the environment- a traveled road by many developed countries- cannot be whatsoever neglected. The hydrophobic nature of dioxins causes them to accumulate in fatty foods, so, dioxins’ levels in other dairy products like pasteurized butter, cheese and cream should be evaluated in future studies. The authors believe that one of the most cost-effective and practical ways to fulfill the requirements of food legislation in reference laboratories is bio-analytical approaches like DR_CALUXR® to regularly monitor samples’ health on a large scale.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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