The survey of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls levels in pasteurized cow′s milk collected in Qazvin

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ABSTRACT

Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDFs), collectively termed dioxins, as well as polychlorinated biphenyls (PCBs) are widespread environmental contaminants. A local survey was carried out on seven samples of pasteurized full-fat grade milk commercially available in Qazvin in 2013 to assess the concentrations of PCDD/PCDFs and dioxin-like PCBs (DL-PCBs) in pasteurized cow′s milk. The mean concentration of PCDD/PCDFs for pasteurized samples determined 0.74 pg toxic equivalent [TEQ]/g fat (range 0.34-1.10). In this survey observed in all samples of pasteurized milk the mean of total TEQ concentration was 0.880 pg TEQ/g fat that lower than the thresholds defined by the European Union regulations for the sum of PCDD/PCDFs and DL-PCBs (P < 0.05). This difference could be possible because of the industrial condition in Qazvin. As a result of the importance of the issue, it is suggested that planning must carry out to control and prevent these types of compounds into the food chain.

1. Introduction

Dioxins are a class of structurally and chemically related polychlorinated aromatic hydrocarbons that mainly includes polychlorinated dibenzo-p-dioxins (PCDDs or dioxins), dibenzofurans (PCDFs or furans) and the “dioxin-like” polychlorinated biphenyls (DL-PCBs). They constitute a group of persistent environmental chemicals and usually occur as a mixture of congeners (1). The largest release of these chemicals today is from open burning of household waste, municipal waste, medical waste, landfill fires, and agricultural and forest fires. Dioxins can be generated and released to the environment from following incineration processes. Dioxin may be absorbed into the body by three main routes of intake: dermal absorption, ingestion, and inhalation. Dioxins compounds are environmentally and biologically stable and, as a result, human exposure is chronic and widespread. From the atmosphere, these particles are deposited and accumulate in the leafy vegetation (such as grass) and in the soil, which acts as a natural sink. DL-PCB concentrations in soil are directly related with the environmental pollution. PCBs presence in soil and vegetation makes these pollutants available to be eaten by animals during grazing, and these contaminants are rapidly absorbed from the gastrointestinal tract. PCBs once absorbed by animals, accumulate in the fat tissue due to their lipophilic nature and during lactation periods they are secreted in milk (2-4).

Dioxins induce a broad spectrum of biological responses, including induction of gene expression for cytochrome P450, CYP1A1, and CYP1A2, disruption of normal hormone signaling pathways, reproductive and developmental defects. Briefly, it indicates that the inappropriate modulation of gene expression represents the initial steps in a series of biochemical, cellular, and tissue changes that result in the toxicity observed.
The toxicity of dioxins are expressed as toxic equivalents (TEQs) where the most toxic congener tetrachlorodibenzo-p-dioxin (TCDD) is rated as 1.0 and the less toxic congeners as fractions of this. When a toxic equivalency factor is multiplied by the congener concentration level, a TEQ value is obtained (5).

People are exposed primarily through foods that are contaminated with PCDDs and PCDFs as a result of the accumulation of these substances in the food chain and in high-fat foods, such as, dairy products, eggs, animal fats, and some fish. Further, the exposure also includes industrial accidents and several miscellaneous exposures. Due to their lipophilicity and their low biodegradability, PCDD/PCDFs and DL-PCBs tend to accumulate in the food chain (6).

In order to actively reduce the presence of dioxins in foodstuffs, threshold is accompanied by measures stimulating a proactive approach such as the use of maximum levels. The maximum level for PCDD/PCDFs in milk and dairy products is 3 pg TEQ/g fat (7). Moreover, the maximum concentration of 6 pg TEQ/g fat has been defined for the sum of PCDD/PCDFs and DL-PCBs and the action level is 2 pg TEQ/g fat for milk and dairy products for DL-PCBs (8).

Given the importance and role of dioxins in the human health risk, many countries to determine their exposure to these compounds in the safety risk assessment programs are at the national level. Analysis of 24 samples of raw and pasteurized milk in Qazvin aimed to profile the regional distribution of PCDD/PCDFs and PCBs and codify of the national standard and threshold’s dioxins.

Searching for relevant articles, a similar study was not found for comparison with the results in the country. For the first time in this study to evaluate dioxin levels in pasteurized milk were consumed in Qazvin.

2. Materials and methods

2.1 Sampling

Seven samples of pasteurized full-fat grade milk commercially available in Qazvin city were collected in 2013. Samples (about 1 L) were stored in glass recipients, frozen at −20°C and then shipped on dry ice to the laboratory for the analysis by high-resolution gas chromatography/mass spectrometry (HRMS) determination (9,10), according to the national standard of Iran with no. 8262/2005.

2.2 Analytical procedures

The analyses on milk samples were conducted at the laboratory of supported with Food and Drug Organization (FDO) of the Islamic Republic of Iran, using an HRMS (Varian 380) method according to the national standard of Iran with no. 8262/2005.

All analytical-reagent grade standards for HPLC (Merck, Germany) and reference materials with 99.9% purity (Siemens Corporate America) were purchased. Seven PCDD congeners (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpC, OCDD), 10 PCDF congeners (2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF), and DL-PCBs congeners (PCB 0,PCB 3, PCB 8, PCB 18, PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180) were measured using gas chromatography coupled with HRMS.

It is centrifuged 30 ml of raw milk at a speed of 2500 rpm for 10 min and cream was transferred to the pot that contains 6 g of sodium sulfate and was added 30 ml n-hexane and was homogenized for 10 min. N-hexane layer through a layer of fiberglass that is coated with sodium sulfate was flat. N-hexane was evaporated at 35°C for approximately 1 ml with a rotary evaporator and was dried the residue with a gentle stream of nitrogen. Extraction of residues determined according to the national standard of Iran with no. 8262-2/2005.

The extract concentrated and re-dissolved in light petroleum. The solution using alkaline aluminum oxide (aluminum oxide was added water and was shaken until all the balls disappeared then allow to rest for at least 24 h when the water in the final product was 90%. It is measured product activity with adding the standard solution β-hexachlorocyclohexane (β-HCH) to made recycle yield 99%), was column chromatography. After washing liquid was condensed to measuring with gas chromatography. Characteristics of chromatography are as follows:

- Column: Capillary column with a DB-1 fused stationary phase (length: 25 m, internal diameter: 0.32 mm, film thickness: 1 µ), column temperature: 90°C, isothermal for 3 min, programmed temperature increase of 35°C/min from 90°C to 160°C, 160°C isothermal for 1 min programmed temperature increase of 2°C/min from 160°C to 220°C and 5°C/min from 220°C to 240°C, for 10 min.
- Site of injection: temperature of 250°C, splitless injection (1 min), electron capture: temperature of 300°C. The intensity of gas: helium carrier gas, inlet pressure 0.8 × 10^5 Pa.
- Nitrogen gas cleaner: 35 ml/min. To confirm founded gas chromatography was performed thin layer chromatography. Diagnosis is based on two factors: the amount of visible reactions and
rheumatoid factor (RF). Pesticide standard solution is spotting on the side of the extracted samples to eliminate RF reproducibility problem.

2.3 Statistical analyses

Analytical procedures yielded three synthetic figures per sample: the concentrations of PCDD/Fs, DL-PCBs, and the total TEQ concentration (the sum of PCDD/PCDFs and DL-PCBs).

Results were expressed in pg TEQ/g fat. Statistical analyses of the data were made using ANOVA of software SPSS version 16, Inc, Chicago, IL, USA. Differences between means were determined by t-test at a level of 0.05. They were interpreted according to the thresholds recommended by the European Union regulations (1,7,10).

3. Results

In this study, seven pasteurized milk samples were examined, and the amount of 7 PCDD congeners, 10 PCDF congeners, and 11 DL-PCBs congeners were determined, which are shown in table 1.

Table 1. Average polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans, dioxin-like polychlorinated biphenyls and total toxic equivalent concentration in pasteurized milk samples (pg TEQ/g fat)

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>DL-PCBs (95% CI)</th>
<th>PCDD/PCDFs (95% CI)</th>
<th>Total TEQ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.110</td>
<td>0.66</td>
<td>0.77</td>
</tr>
<tr>
<td>2</td>
<td>0.080</td>
<td>1.06</td>
<td>1.14</td>
</tr>
<tr>
<td>3</td>
<td>0.013</td>
<td>0.83</td>
<td>0.84</td>
</tr>
<tr>
<td>4</td>
<td>0.050</td>
<td>0.37</td>
<td>0.42</td>
</tr>
<tr>
<td>5</td>
<td>0.020</td>
<td>0.34</td>
<td>0.36</td>
</tr>
<tr>
<td>6</td>
<td>0.360</td>
<td>1.10</td>
<td>1.46</td>
</tr>
<tr>
<td>7</td>
<td>0.330</td>
<td>0.82</td>
<td>1.15</td>
</tr>
</tbody>
</table>

P < 0.05, n = 3. DL-PCBs: Dioxin-like polychlorinated biphenyls; PCDDs: Polychlorinated dibenzo-p-dioxins; CI: Confidence interval; PCDFs: Polychlorinated dibenzofurans; TEQ: Toxic equivalent.

The total concentrations of DL-PCBs for pasteurized milk samples ranged between 0.02 pg TEQ/g fat and 0.36 pg TEQ/g fat with an average value of 0.137 pg TEQ/g fat for the samples 5 and 6, respectively (Table 2).

Table 2. Average polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans, dioxin-like polychlorinated biphenyls and total toxic equivalent concentration in pasteurized milk samples (pg TEQ/g fat)

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Sample no.</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCDD</td>
<td>Pasteurized</td>
<td>7</td>
<td>0.690</td>
<td>0.3130</td>
</tr>
<tr>
<td>PCDF</td>
<td>Pasteurized</td>
<td>7</td>
<td>0.055</td>
<td>0.0178</td>
</tr>
<tr>
<td>PCDDF</td>
<td>Pasteurized</td>
<td>7</td>
<td>0.740</td>
<td>0.3000</td>
</tr>
<tr>
<td>PCB</td>
<td>Pasteurized</td>
<td>7</td>
<td>0.137</td>
<td>0.0400</td>
</tr>
<tr>
<td>TEQ</td>
<td>Pasteurized</td>
<td>7</td>
<td>0.880</td>
<td>0.3200</td>
</tr>
</tbody>
</table>

P < 0.05, n = 3. PCDD: Polychlorinated dibenzo-p-dioxin; PCDF: Polychlorinated dibenzofuran; PCDDF: sum of Polychlorinated dibenzo-p-dioxin & Polychlorinated dibenzofuran; PCB: polychlorinated biphenyl; TEQ: Toxic equivalent.

The total concentrations of DL-PCBs for pasteurized milk samples ranged between 0.02 pg TEQ/g fat and 0.36 pg TEQ/g fat with an average value of 0.137 pg TEQ/g fat for the samples 5 and 6, respectively (Table 2).

In the pasteurized milk samples lowest average value for the total TEQ concentration was observed for the sample 5: 0.39 pg TEQ/g fat, whereas the highest value was obtained for the sample 6: 1.46.58 pg TEQ/g fat (Table 2).

Table 1 shows in all samples of pasteurized milk the mean of total TEQ concentration was 0.880 (pg TEQ/g fat) that lower than the thresholds defined by the European Union regulations for the sum of PCDD/PCDFs and DL-PCBs (6 pg TEQ/g fat) (P < 0.05).

In the present survey, there was a lower of PCDD/PCDFs levels separately and in sum of PCDD/PCDFs and DL-PCBs thresholds established by Commission Recommendation 2006/199/EC (P < 0.05).

As shown in figure 1, respectively, among the milk samples of pasteurized milk the highest concentration of PCDD congeners was 2,3,7,8-TCDD congener. Pattern of PCDFs confirms that in pasteurized milk the concentration of 0.74 pg TEQ/g fat (Table 2) for the samples 5 and 6, respectively.

![Figure 1](image-url). Mean polychlorinated dibenzo-p-dioxins profiles in pasteurized milk samples (pg TEQ/g fat) (P < 0.05, n = 3).
samples 2,3,7,8-TCDF congener were higher extent than congeners (Table 3).

Table 3. Mean polychlorinated dibenzofurans profiles in pasteurized milk samples (pg TEQ/g fat)

<table>
<thead>
<tr>
<th>Congeners</th>
<th>Mean (pg TEQ/g fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TCDF</td>
<td>0.028</td>
</tr>
<tr>
<td>1,2,3,7,8-OCDF</td>
<td>0.000</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDF</td>
<td>0.020</td>
</tr>
<tr>
<td>1,2,3,7,8-HxCDF</td>
<td>0.000</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDF</td>
<td>0.010</td>
</tr>
<tr>
<td>2,3,4,6,7,8-HxCDF</td>
<td>0.010</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDF</td>
<td>0.001</td>
</tr>
<tr>
<td>1,2,3,4,7,8,9-HpCDF</td>
<td>0.000</td>
</tr>
<tr>
<td>OCDF</td>
<td>0.000</td>
</tr>
</tbody>
</table>

P < 0.05; n = 3

4. Discussion

PCDD, PCDF, and DL-PCB, can inter the air and can deposit on feed in certain conditions, such as vegetables cultivated on fields (corn, oat) or already manufactured and stored at the farms (silage).

This study determined PCDD, PCDF, and DL-PCB in examined pasteurized milk, and it can be due to their lipophilic nature for the possible presence of dioxins in the fat part of milk products. Milk fat has more amounts of these chemical components. Thought dairy products are the corruptible products, and their biological and chemical contamination may be high. The mean TEQ value including PCDD/PCDFs to examined pasteurized milk samples that they were 0.74 pg TEQ/g fat and lower of PCDD/PCDFs levels separately and sum of PCDD/PCDFs and DL-PCBs thresholds established by Commission Recommendation 2006/199/EC (P < 0.05). For this reason, it is recommended that people use pasteurized milk, it has health licenses from the FDO till does not develop food poisoning.

A similar study is conducted by Focant et al. that analyzed of 7 PCDDs, 10 PCDFs and 4 PCBs on congener-specific in eight different brands commercial long-life pasteurized cow’s milk available in Belgium.(9) It is observed the toxic equivalent value for PCDD/PCDFs in all analyzed milks was 2.23±0.55 pg TEQ/g fat, which is below the recommended EU threshold value of 3 pg TEQ PCDD/PCDFs/g of milk fat. The mean TEQ value of PCBs was 1.09±0.30 pg TEQ/g fat.

Since processing and heating have not effect on the reduction of dioxins, this different can relate to contamination concentrations of raw milk.

Ramos et al. were analyzed 23 samples of pasteurized cow’s milk for PCDD in 1997. PCDD concentrations ranged from 1.3 to 2.47 pg TEQ/g basis (11).

The mean TEQ value, including DL-PCBs to examined pasteurized milk samples that was 0.137 pg TEQ/g fat and it is similar with results of Luzardo et al., that they measured concentrations of DL-PCBs in conventional milk samples from the Canary Islands market (Spain). The result of the analysis showed that median concentrations-PCBs was 0.10 (pg WHO-TEQs/g fat) (12).

These findings are of concern due to the deleterious health effects (neurobehavioral, reproductive, immunologic, and carcinogenic) which have been attributed to dioxin-like substances. Some evidences suggest that even low doses of DL-PCBs can cause subtle effects during prolonged exposure if the exposure occurs during prenatal and postnatal development especially. In this sense, there has been increasing concern regarding the effects on children’s neurological development (13).

5. Conclusion

The present study indicated that these compounds levels in pasteurized milk available in Qazvin were lower than the maximum limit imposed by Regulation (EC) 199/2006.

However, a comprehensive monitoring of PCDD/PCDFs and DL-PCBs in the environment as well as in feed and food is still necessary for other parts of the country to protect public health. Further actions to control the potential sources of environmental contamination by these pollutants, may need to be supplemented by measures to prevent direct contamination of feeding stuff or food and setting of a national standard and threshold’s dioxins to reduce general population exposure.

Conflict of Interests

Authors have no conflict of interest.

Acknowledgments

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References


