THE ANALYSIS OF PERCHLORATE IN DAIRY PRODUCT SAMPLES

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Introduction

Perchlorate is nowadays well known as emerging contaminant due to its potential adverse effects on human thyroid function like hyperthyroidism by competitively inhibiting iodide transport[1]. Moreover, perchlorate can affect thyroid function of newborns by reducing T3 hormone which is required for normal development of central nervous system in fetuses and infants[2]. According to U.S. FDA results, the concentration of perchlorate in milk was the highest compared to the other foods[3]. It is known that the main exposure route of perchlorate is food ingestion[4] and the milk is main meal for young children, so, they are more susceptible to harmful effect of perchlorate than adult. Furthermore, the exposure of pregnant women to perchlorate can affect their babies and individuals with compromised thyroid function can have detrimental effect. Until now, nationwide monitoring of perchlorate contamination in foods including milk and yogurt has not been performed yet in Korea and there is no national perchlorate guideline for food. Therefore, we measured perchlorate in milk and yogurt samples to understand the level of perchlorate contamination in dairy products and to assess the exposure of perchlorate in Korean human.

Materials and Methods

Materials

A certified perchlorate analytical standard solution was purchased from AccuStandard (New haven, CT, USA). The $^{18}O_4$-labeled perchlorate as internal standard (IS) was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Acetonitrile and HPLC grade water were purchased from J.T. Baker (Phillipsburg, NJ), 40% methylamine solution from Yakuri pure chemicals co, Ltd (Japan) and acetic acid was from Waco Pure Chemical industries, Ltd. (Japan).

Analytical Method

24 milk samples and 20 yogurt samples were randomly purchased in Korean market and stored at below 4℃ prior to extraction. Samples were extracted and analyzed within 7 days. The preparation of perchlorate in dairy product samples was conducted by modified US FDA method. 5 mL milk aliquots were pipetted in 50mL polypropylene conical tubes. Surrogate standard ($Cl^{18}O_4^-$) was spiked into the samples directly and extracted with 5 mL of 1% acetic acid in water and 20 mL of acetonitrile and mixed by shaker (200rpm, 2hr). After centrifugation (4000rpm, 1hr), the supernatant was passed through Supelclean™ Envi™-Carb SPE cartridge (Bellefonte, PA, USA). These cartridges were prewashed sequentially with 6mL of acetonitrile and 6mL of 1% acetic acid in water. The final volume of sample extract was adjusted to 40mL with reagent water and the sample was filtered with 0.20 μm pore size nylon syringe filter prior to LC-MS/MS. The 1% acetic acid solution helps to prevent retention of perchlorate on the SPE cartridge and acetonitrile makes the sample less cloudy[5]. Perchlorate was analyzed using a 1200 HPLC equipped with a 6460 triple-quadruple tandem mass (Agilent, USA) in electrospray negative mode. A 50 μL aliquot of the final sample was injected into the AS21 column (4.6 mm i.d. X 150 mm length, 5 μm; Dionex technology) and 231 mM methylamine solution was used as mobile phase. Perchlorate calibration curve was prepared with ten different levels of perchlorate standard solutions (0.10, 0.25, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100μg/L). Detailed analytical condition was shown in Table 1.

QA/QC

The recoveries of this analytical method were measured in milk and yogurt samples after fortifying perchlorate (20 ug/L) for three times. The recoveries of perchlorate in milk and yogurt samples were 114.7% and 118.3% (RSD 0.52%, 2.32%), respectively. Method of detection limit (MDL) and limit of quantitation (LOQ) were obtained from the EPA method 6850. The MDL was calculated by Equation (1) with injection of seven replicates and the LOQs were defined as 10 times of standard deviation associated with seven replicate analyses. The calculated MDL and LOQ were 3.26 μg/L and 2.25 μg/L.

Eqn (1) $\mathrm{MDL} = ts$
Where, t=3.14 for six degrees of freedom and s is the standard deviation of the quantified perchlorate concentration in the seven replicate injections.

Results and Discussion

Concentrations and distributions of perchlorate in dairy product samples

44 different dairy product samples purchased from Korean market were analyzed for perchlorate. The perchlorate levels found in these dairy products were summarized in Table 2 and measurable amounts of perchlorate (over LOQ) were detected in all of samples. The concentrations of perchlorate in five types of dairy products (whole milk, low fat milk, processed milk, curd type yogurt and liquid type yogurt) ranged from 2.44 to 21.9 μg/L and the average concentration of perchlorate in dairy products was 7.96 μg/L. The highest concentration of perchlorate was found in liquid type yogurt, whereas the lowest was found in processed milk (Figure1). Even though the highest concentration was observed in liquid type yogurt but the average concentration was lowest. According to Shi et al. (2007), the main source of perchlorate in milk may be forage and water hat that are fed to the cattle[6].

Comparison of perchlorate levels between other studies

The perchlorate levels in milk samples were reported from China, Japan and USA and the average value of perchlorate in this study was similar with other countries but had a wide range (Figure 2). Dyke et al analyzed the perchlorate in dairy milk samples from 48 different locations in Japan[7] (range from 5.5 to 16.4 μg/L; mean 9.4 μg/L) and US Food and Drug administration reported the average concentration in milk samples was 5.76 μg/L (range from 1.5 to 11.3 μg/L). In China, Shi et al analyzed perchlorate in 17 milk samples collected in Beijing (range from 0.3 to 9.1 μg/L; mean 2.0 μg/L)[6].

Estimated Dietary Exposure to perchlorate from dairy products

We estimated dietary perchlorate dose from dairy product samples with perchlorate concentrations analyzed in this study. Dietary perchlorate dose was the estimated using the following equation.

$$\text{Perchlorate dose (μg/kg bw)} = \frac{7.96\ (\mu g/L) \times \text{Dairy products consumption (g/day)}}{1.04\ (kg/L) \times \text{Body weight (kg bw)} \times 1000\ (g/kg)}$$

Where, 7.96 is the average concentration of dairy products in this study (μg/L), 1.04 is the specific gravity for milk (kg/L), 1000 is the conversion constant (g/kg). Dairy products consumption and body weight data were obtained from the Korea National Health and Nutrition Survey (Ministry of Health & Welfare, 2000). The estimated perchlorate diary exposure from dairy products according to age groups is listed in table 3. For all age group, potential exposure to perchlorate was below the reference dosage (0.7 μg/kg) proposed by the National Academy of Sciences (NAS) but the perchlorate exposure of children is pretty higher than adult. The NAS reference dosage (0.7 μg/kg) is the reduced no-observed effect level (NOEL, 7 μg/kg) which a 10-fold uncertainty factor was applied to address all potentially sensitive subpopulations[8]. Thus, we can conclude that the exposure to perchlorate by consuming dairy products in Korean is satisfied with NAS guideline but higher exposure to perchlorate in infant was observed. Therefore, further investigation of perchlorate in other food samples is needed for exact assessment of perchlorate exposure in young age group.

Acknowledgements

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Table 1 Source parameters.

Parameters	Conditions
Column temp.	30 ℃
Ionization mode	ESI negative
Gas temp.	350 ℃
Gas flow	8 L/min
Capillary vol.	1.50 kV
Nebulizer	40 psi
Sheath gas temp.	300℃
Sheath gas flow	10 L/min

Table 2 Concentrations of perchlorate found in samples.

Commodity	n	Mean	Median	Conc. Range
Whole milk	10	7.40	7.58	5.15~8.81
Low fat milk	3	11.0	10.1	5.35~17.4
Processed milk	11	7.49	5.81	2.44~15.9
Yogurt (curd)	9	9.58	8.90	5.36~14.7
Yogurt (liquid)	11	6.79	6.24	2.60~21.9

Figure 1 Distribution of perchlorate concentration found in milk samples.

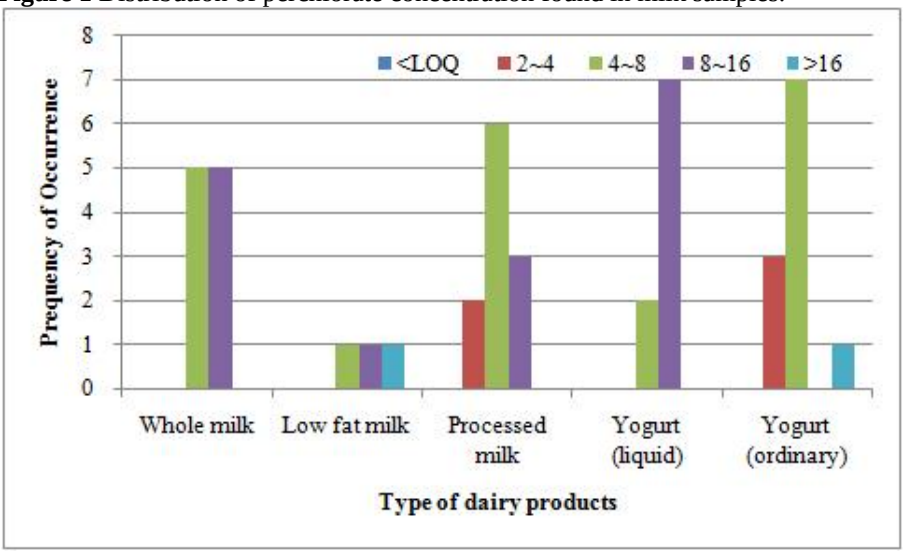
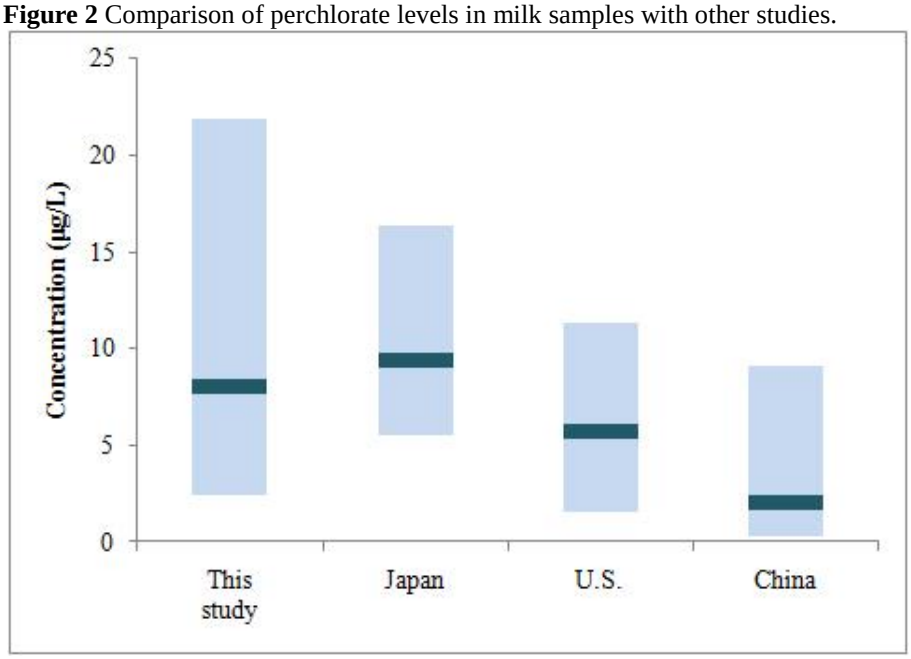


Figure 2 Comparison of perchlorate levels in milk samples with other studies.



* Japan: Dyke et al. (2007); U.S.: U.S. FDA (2007); China: Shi et al. (2007).

Table 3 Perchlorate exposure of children and adults whom consume dairy products.

Consumer age:	1~2	3~6	7~12	13~19	20~29	30~49	50~64	65 and older
Dairy products consumption (g/day)								
males	329.4	193.0	200.0	146.5	63.8	46.9	30.5	25.7
females	317.6	173.0	176.7	101.8	71.7	58.3	36.7	30.8
Body weight (kg bw)								
males	11.0	17.4	37.6	64.1	70.4	70.1	66.2	61.2
females	10.8	16.7	35.3	53.8	54.4	58.0	59.2	53.1
Exposure (μg/day)								
males	2.52	1.48	1.53	1.12	0.49	0.36	0.23	0.20
females	2.43	1.32	1.35	0.78	0.55	0.45	0.28	0.24
Dose (μg/kg bw)								
males	0.23	0.09	0.04	0.02	0.01	0.01	0.00	0.00
females	0.23	0.08	0.04	0.01	0.01	0.01	0.00	0.00
Dose/Reference dosage (NAS, 0.7μg/kg bw)								
males	32.6%	12.1%	5.8%	2.5%	1.0%	0.7%	0.5%	0.5%
females	32.2%	11.3%	5.5%	2.1%	1.4%	1.1%	0.7%	0.6%