Perchlorate in Milk

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Perchlorate was unambiguously detected by ion chromatography-suppressed conductivity (IC-CD) and/or ion chromatography−electrospray mass spectrometry (IC-MS) in seven of seven supermarket milk samples bought randomly in Lubbock, TX. Quantitation by IC-MS and IC-suppressed conductivity detection in conjunction with a preconcentration-preelution method provided comparable results. With a sample cleanup procedure that involved protein removal by ethanol and sequential passage though activated alumina and C-18 silica, the limit of detection for perchlorate in milk was 0.5 μg/L. The levels found ranged from 1.7 to 6.4 μg/L. An evaporated milk sample contained perchlorate at 1.1 ± 0.6 μg/L level, while we did not find detectable levels in a reconstituted powdered milk sample.

In recent years the concern about perchlorate in drinking water has become such a major public issue that lengthy articles have appeared in the *Wall Street Journal* (*1*), even as a front page story. Perchlorate disrupts thyroid function by competitively inhibiting iodide transport (*2*). The resulting malfunction of the $Na^{+}-I^{-}$ Symporter (NIS) (*3*) reduces thyroid hormone production (*4*) and can impair the development of the gland. Pregnant women, children, and people with compromised thyroid function are thus particularly at risk. Although the EPA is yet to set a specific drinking water limit, the State of California has already adopted an action level of 4 μg/L (*5*) based on the draft toxicology and risk assessment review (*6*).

Since it is difficult to use the EPA recommended method (*7*) for the analysis of trace levels of perchlorate in high salinity waters or other challenging samples, a preconcentration-preelution (PC-PE) method was recently developed at this institution that simplifies sample cleanup/enrichment and the analysis (*8*). This method was then used for the first analysis of perchlorate in food. Colorado river water is known to contain trace levels of perchlorate and is widely used for irrigation. At the request of the Environmental Working Group, a public interest organization, we analyzed 22 samples of lettuce purchased in Northern California. Four of the 22 samples contained quantifiable levels of perchlorate. The highest concentration found was 121 μg/kg (wet weight) (*9*). This was an exploratory rather than a definitive study. However, others have since reported finding perchlorate in 18 out of 18 samples in California lettuce (*10*). Such results are in fact supported by earlier studies from the EPA (*11*). The levels of perchlorate found in lettuce may or may not represent an exposure level of concern; at the present time the EPA is not at liberty to address issues related to toxicology aspects of perchlorate contamination (*12*).

TABLE 1. Concentrations of Perchlorate Found in Supermarket Milk Samples, Lubbock, TX

description	IC-conductivity laboratory A μg/L ± SD	IC-MS laboratory B μg/L ± SD	container type and size
brand A	6.30 ± 1.40	6.23 ± 1.63	paper, quart
brand B	4.10 ± 0.44	5.68 ± 2.71	plastic, pint
brand C	1.75 ± 0.39	1.81 ± 0.25	plastic, 1/2-gallon
brand D[c]	4.89 ± 1.0	4.96 ± 0.67	paper, 1/2-gallon
brand E	4.03 ± 0.90	6.36 ± 2.78	plastic, 1/2-gallon
brand F	4.91 ± 1.40	NA[a]	glass, quart
brand G[c]	1.87 ± 0.04	NA[a]	paper, quart
evaporated milk	1.12 ± 0.60	NA[a]	can, 12 oz.
powdered milk	ND[b]	NA[a]	plasticized paper pouch[e]
processed blank	ND[b]	ND[b]	

[a] NA, not analyzed. [b] ND, not detected. [c] Labeled "organic". [d] Vitamin D not added. [e] Instructions call for dissolving the contents to make 1 quart of milk, this was followed.

We investigated the occurrence of perchlorate in milk; it is obviously a very relevant matrix for young children. An NIS also exists in mammary tissue concentrating iodine in milk to ensure availability of this essential nutrient to infants (*13*). As with the thyroid NIS, perchlorate is likely to be taken up by the mammary NIS and be expressed in milk. Maternal exposure to perchlorate from all sources is important as this leads to exposure of the developing fetus. Milk is an important dietary ingredient for expecting and lactating mothers. This correspondence reports the levels of perchlorate found in milk samples randomly bought from supermarkets in Lubbock, TX.

Materials and Methods

Perchlorate calibration standards and spikes for milk samples were prepared from a 100 μg/mL certified $NaClO_4$ standard (AccuStandard, Inc., New Haven, CT) in distilled, deionized Milli-Q (MQ) water. Five-milliliter milk aliquots were pipetted in 15 mL prewashed polypropylene Falcon tubes, and 10 mL of 200-proof chilled (5 °C) ethanol was added and mixed by repeated inversion. The samples were then allowed to stand for 24 h refrigerated (~4 °C). After centrifugation (3700 rpm, 20 min at −5 °C), the supernatant was collected in a clean 15-mL Falcon tube. One gram of Activated alumina (DD6, Alcoa, Port Allen, LA) was added to each tube, the suspension was mixed by inversion, and the samples were refrigerated for an additional 24 h. Each sample was then evaporated to ~4 mL by warm nitrogen evaporation and then processed through C-18 SPE cartridges (Fisher). These cartridges were prewashed sequentially with 2 mL of *n*-hexane, 2 mL of acetone, and 2 mL of MQ water. The evaporated sample was passed first through a 2 g C-18 SPE cartridge, and the effluent was then passed through a second C-18 SPE (1 g) cartridge. The samples acquire some extra liquid from the SPE cartridges. After the C-18 cleanup, they were placed in the nitrogen evaporator, and then the requisite amount of MQ water was added as needed to return the samples to the original volume of 5 mL. MQ water blanks in quadruplicate were taken through the entire sample processing procedure. Milk samples, spiked to contain 5 and 10 μg/L of additional perchlorate, were also processed similarly.

Analyses of the processed sample by IC-suppressed conductivity were carried out in our laboratories at the

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Institute of Environmental and Human Health on a DX-320 IC System equipped with a GP50 gradient pump, an LC-25 oven, a CD20 conductivity detector, an ASRS-Ultra electrodialytic suppressor (operated in the external water mode), and an AS40 autosampler (all from Dionex Corp.). PeakNet 6.2 chromatography software was used for system control and area-based analyte quantitation using the external standard mode and a 6-point (plus blank) calibration curve. The following changes were made from the previous protocol (*8*): The temperature was maintained at 30 °C. For PC-PE, 2 TAC-LP1 (4 × 35 mm) columns were used. The sample (1 mL loop) loading and preelution were carried with 10 mM NaOH at 0.4 mL/min for 6.2 min and then chromatographed on AG11 (4 × 50 mm) − AS16 (4 × 250 mm) columns with 100 mM NaOH at 1.0 mL/min. For an aqueous standard, this system has a $S/N = 3$ limit of detection of 0.4 μg/L.

Confirmatory analyses by IC-mass spectrometry were conducted in our laboratories at the Department of Chemistry. The IC front end used a DX-600 chromatograph with a CD25 conductivity detector and a Finnigan AQA mass spectrometer. A single TAC LP1 column was used for PC-PE (10 mM NaOH, 2.5 mL/min), with AG16/AS16 being used for separation. A 1-mL injection of the processed sample was used. The mass spectrometer was operated in the electrospray mode (3 kV), an injection temperature of 340 °C, and an ionization voltage of −40 V. After confirming that the peaks for ClO_4^- do occur in the samples at m/z 99 and 101 (due respectively to $_{17}Cl^{35}$ and $_{17}Cl^{37}$) at the previously determined perchlorate retention time and approximately in the expected 3:1 isotopic ratio, quantitation was done on the basis of the greater intensity signal at m/z 99.

Milk samples were procured from local supermarkets on different dates. Samples A−G were all standard regular milk (not low-fat type), fortified with vitamin D (except as noted). The seven milk samples tested represented six different brands, essentially all the brands of milk available in this city. In both laboratories, the same sample was processed separately at least three times, and three replicates were thus analyzed in each laboratory. The analyses were conducted blind.

Results and Discussion

The significant occurrence of perchlorate in all milk samples analyzed at levels that are comparable or even greater than the current California "action level" for the concentration of perchlorate in drinking water came as a considerable surprise to us. While it is possible that some of the brands have ultimately the same source of the milk (four of the six brands tested were locally bottled at the same plant, identified on each container), all of the purchased supermarket milk did not come from the same source (two were bottled in separate plant locations outside the State of Texas). Based on this limited study, it is not clear how widespread perchlorate contamination of milk may be, but clearly such a study is warranted. There are no extant regulations that govern the perchlorate concentration of feedwater in dairy operations.

The close comparability of the results between two independent methods at two separate laboratories and the unambiguous mass spectrometric identification leaves little doubt about the occurrence of perchlorate in the samples and the quantitative accuracy of the results within the stated limits. Recovery of perchlorate through the entire procedure including the sample cleanup steps was studied by the IC-conductivity method and found to be generally excellent. Both at 5 and 10 ppb, the recoveries ranged from 96 to 102%. Only the 10 ppb spike in the brand E samples showed a lower recovery (82.5%). The sample preparation procedure and the PC/PE IC method provided a limit of detection (LOD) of 0.5 μg/L of perchlorate in the original sample. Without the PC/PE method, the LOD was no better than 20 μg/L.

While this investigation demonstrates that perchlorate is indeed excreted in milk, further research is necessary to determine the extent of perchlorate contamination of commercial milk in a study of much larger scope as well as the dose-excretion relationship.

To assess other sources of perchlorate intake, we looked at the perchlorate levels of the local tap water. While we have not as yet studied the seasonal variations in detail, the concentrations vary from below the LOD (0.50 μg/L) to above 4 μg/L, with a mean value of 2.5 ± 1.1 μg/L in the samples in which it exists above the LOD. While it should not be construed as a generality, we note in passing that in an identical analysis protocol, we found 3−4.5 μg/L of perchlorate by the two methods in a milk sample from a single human volunteer from this locality.

In addition to direct exposure to infants through contaminated breast milk, prenatal exposure may occur via maternal exposure to perchlorate, not only through drinking water but also via various food substances, including lettuce and milk/milk products. This has the potential to affect early gestational development by altering maternal thyroid function, altering levels of fetal exposure to maternal thyroid hormones, and altering the fetal thyroid (*14*). Thyroid hormone deficiency is of particular concern during development as these hormones are known to regulate the development of the brain (*15*).

In devising control and regulation strategies for perchlorate, the total exposure must be considered. Drinking water is only a limited part of this exposure. The total exposure cannot be estimated until we have a better quantitative knowledge of the extent of occurrence of perchlorate in various types of food. This is not a simple task as appropriate digestion/extraction procedures must first be developed before bioaccumulation of perchlorate can be assessed. Traditional acid digestion procedures are not applicable in determining perchlorate. We hope that increased awareness of food and drink as vectors for perchlorate intake will result in an increased willingness to act on it. Potential existence of perchlorate in produce has been known for sometime. Official commitments were made to study the extent of the problem, but in the end no significant assessment effort resulted (*16*).

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