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# METHOD EVALUATION TO MEASURE PERSISTENT BIOACCUMULATIVE TOXIC POLLUTANTS IN COW MILK

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## Introduction

The US EPA estimates that approximately 35% of an adult's daily intake of dioxins is derived from dairy products. The percentage for children is even higher. Persistent, bioaccumulative and toxic pollutants (PBTs), including dioxins, bioaccumulate through the food chain and ultimately result in low-level contamination in most animal fats. It is important to understand the PBT levels in milk, as milk fat may be one of the highest dietary sources of PBT exposure. Analysis of milk also allows the opportunity to investigate geographic variability, as milk is produced and distributed on a regional scale.

The work presented here was the result of a study funded by the U. S. Environmental Protection Agency's (EPA) National Center for Environmental Assessment (NCEA). The goal was to analyze regional milk samples collected from the EPA Environmental Radiation Ambient Monitoring System (ERAMS) collection sites for a wide variety of PBTs, including pesticides, polyaromatic hydrocarbons (PAHs), polychlorinated naphthalenes (PCNs), brominated diphenyl ethers (BDEs), chlorinated dibenzodioxins, chlorinated dibenzofurans, polychlorinated biphenyls, lead, and cadmium. To determine the regional differences in the milk, the 46 sampling stations located within major population centers of 41 states and Puerto Rico were grouped into eight regions. To determine seasonal differences, milk was analyzed from a summer collection and from a winter collection. The results of this study were originally published in Schaum et al. (2003). This paper focuses on the chemical analysis of a subset of the chemicals addressed in the original study: pesticides, PAHs, BDEs, and PCNs.

A literature search was conducted to ascertain relevant sample preparation and analytical methods for organic analytes in milk or high-fat content samples. The resulting methods were evaluated for applicability to the analytes of interest, reported analyte recoveries and ease of implementation. Spike recovery tests were conducted with locally purchased store-bought whole milk using the most promising methods. As difficulties were encountered in the application of the methods, modifications were instituted to accommodate the differences in equipment or to improve recovery of the analytes. Descriptions of these final methods are discussed here.

## Materials and Methods

The ERAMS milk collection network was used to collect samples in July 2000 (44 separate milk samples) and January 2001 (45 separate milk samples). Freezing prior to shipment to the analytical laboratory preserved the samples. The samples were thawed and combined to prepare the regional composite samples. Equal amounts of the 4-8 individual samples in a geographical region were combined to prepare the composite samples. Grand composite samples were also

prepared by combining amounts from each individual sample adjusted on the basis of relative milk production represented by each ERAMS station.

Analysis of whole milk samples (regional or grand composite samples) for eight PAHs and four organochlorine pesticides (mirex, endrin, pentachlornitrobenzene, and pentachlorophenol) was carried out following a method developed for pesticides and PAHs in food matrices[1]. The method entailed homogenizing a 25-mL aliquot of milk with dichloromethane. The sample was centrifuged and the liquid layers were transferred to a separatory funnel. The dichloromethane was drained through a bed of sodium sulfate to remove residual water, and then concentrated. The dichloromethane extract was applied to a gel permeation chromatography (GPC) column; the first 30 minutes of eluent from the GPC column containing fats was discarded. The remainder of the eluent was collected and concentrated. The sample was applied to a florisil solid phase extraction (SPE) column. The SPE eluent was concentrated and analyzed using a gas chromatograph/mass spectrometer in the multiple ion mode (GC/MS/MID). In the method demonstration experiments, recoveries of pesticides and PAHs spiked into control milk averaged 76-143% and 83-130%, respectively.

A single method was used for detection of both the BDEs and PCNs. Two sets of samples (one for BDEs and one for PCNs) were prepared following a method developed for determining chlorinated dibenzodioxins and furans from milk[2], which included slight modifications to the fractionation on the carbon/silica column. BDEs and PCNs were spiked into 300 mL of whole milk at 50 ng and 5 ng, respectively, for a spike level of 170 pg/mL and 17 pg/mL in milk. Seven target BDEs were included in this study including BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183. The three target PCNs were PCN 36, PCN 52, and PCN 66. The PCNs eluted in Fractions 1 and 2 and the BDEs eluted in Fractions 2 and 3 from the carbon/silica column clean up. From milk spiked with the BDEs, Fractions 2 and 3 from the carbon/silica column for each sample (~100 µL each) were combined, the solvent was exchanged into hexane, and chromatographed on an alumina column. This final extract was analyzed using GC/MS. The BDE recoveries ranged from 77-108% in the whole milk extracts and 70-94% in the solvent spike. Fractions 1 and 2 from the carbon/silica column for each PCN-spiked milk were analyzed separately using low resolution gas chromatograph equipped with a mass selective detector (GC-MSD) operating in selected ion mode (SIM).

**Results and Discussion**

It was noted during the sample thawing prior to sample compositing that significant amounts of fat congealed. The samples were vigorously shaken to break the congealed fat to obtain a homogenous sample. The amount of fat globules varied from sample to sample suggesting varying amounts of fat in the milk. The lipophilic compounds evaluated in this study were assumed to partition completely into the milk fat. Due to the nonhomogenous nature of the milk sample, the concentrations of the analytes may have been negatively biased. This problem may be alleviated by adding a homogenizing step prior to aliquoting thawed samples or aliquoting the samples for extraction prior to freezing.

The recoveries of the pesticides and PAHs in the laboratory matrix spikes prepared with the field samples ranged from 53-117% and 39-108%, respectively. At detection limits of approximately 100-400 ng/L, none of the pesticides were detected in any field sample. As shown in Table 1, the PAH levels ranged from nondetect to 35 µg/L in the regional samples and from nondetect to 777 ng/L in the grand composite samples. Naphthalene and phenanthrene had the highest grand composite values in both summer and winter. Acenaphthene and benzo(a)pyrene were not

detected in any samples. The New England winter sample contained elevated levels of PAHs. However, the levels of PAHs in the New England summer sample were comparable to that of the other summer regional samples. The PAHs were also consistently higher in the winter samples; PAH levels were also higher in the East and North samples than in the West and South samples.

The recovery results for PCN and BDE in the field samples differed significantly from the recoveries in the method demonstration phase of this work. The PCN recoveries from spiked whole milk samples ranged from 0-100%. The two PCN fractions (fractions 1 and 2 from the carbon/silica column) were not submitted to alumina column clean up because the extract solvent at that point could not be exchanged into hexane for the alumina column method. The PCN recoveries in the solvent spike ranged from 112-116%. Although some BDEs and miscellaneous organics were detected in the field samples (regional or grand composites), the methods used for these analytes and the PCNs are not sufficiently rugged to be used to definitively quantitate the concentration of analytes in the field samples. Further method development is required before additional analyses can be undertaken. The method could likely be improved to give reliable results with little modification.

The potential exposures resulting from the detected PBTs in milk were estimated using the mean of the grand composites and assuming long-term ingestion at these levels. Assuming milk fat content of 3% by weight, the daily intake of the detected PAHs from ingestion of all milk fats was estimated to be 0.6 µg/day.

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**References**

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## Table 1. Concentrations of PAHs in Milk (ng/L)

| Analytes and Detection Limits, ng/L in Milk | Acenaph-thene | Acenaph-thylene | Anthra-cene | Benzo (a) pyrene | Fluorene | Naphtha-lene | Phenan-threne | Pyrene |
|---|---|---|---|---|---|---|---|---|
| | 100 | 30 | 100 | 400 | 30 | 20 | 30 | 40 |
| July 2000 | | | | | | | | |
| New England | ND[1] | ND | ND | ND | 37 | 546 | 166 | 70 |
| Mid-Atlantic | ND | 40 | [46][4] | ND | 182 | 1050 | 646 | 120 |
| South Central | ND | ND | [38] | ND | 51 | 546 | 241 | 76 |
| North Central[2] | ND | ND | ND | ND | 36 | 597 | 148 | 58 |
| West Central | ND | 58 | ND | ND | 53 | 871 | 221 | 79 |
| Southwest | ND | ND | [62] | ND | 535 | 551 | 3247 | 202 |
| Far South | ND | ND | ND | ND | ND | 750 | 209 | 70 |
| Far West | ND | 45 | ND | ND | 58 | 947 | 431 | 99 |
| Grand Composite | ND | ND | [50] | ND | 237 | 552 | 1124 | 174 |
| January 2001 | | | | | | | | |
| New England | ND[1] | 828 | 1404 | ND | 2530 | 3095 | 35944 | 631 |
| Mid-Atlantic | ND | 69 | [64] | ND | 436 | 1099 | 3826 | 296 |
| South Central | ND | 40 | ND | ND | 154 | 856 | 745 | 150 |
| North Central[2] | ND | 46 | [42] | ND | 142 | 992 | 595 | 134 |
| West Central | ND | 59 | [60] | ND | 468 | 1032 | 2896 | 306 |
| Southwest | ND | 57 | [52] | ND | 341 | 829 | 1909 | 126 |
| Far South | ND | BLOQ [2] | [39] | ND | 97 | 583 | 582 | 111 |
| Far West | ND | 40 | [41] | ND | 127 | 882 | 641 | 151 |
| Grand Composite | ND | BLOQ | ND | ND | 120 | 644 | 430 | 108 |
| Grand Composite Mean [3] | ND | ND | 35 | ND | 178 | 598 | 777 | 141 |

[1] Not detected
[2] BLOQ = below the limit of quantitation, indicates that peak seen but could not be estimated.
[3] The grand composite mean is the average of the two seasons.
[4] Concentration is below the limit of quantitation, but the peak appears to be real