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PRODUCTION-RELATED CONTAMINANTS (PESTICIDES, ANTIBIOTICS AND HORMONES) IN ORGANIC AND CONVENTIONALLY PRODUCED MILK SAMPLES SOLD IN THE USA

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Abstract: We sought to determine if contaminant levels differ by the production method used. Half-gallon containers of organic and conventional milk were collected in each of nine US regions and shipped on ice for analysis. Pesticide, antibiotic and hormone (bovine growth hormone (bGH), bGH-associated insulin-like growth factor 1 (IGF-1)) residues were measured using liquid or gas chromatography coupled to mass or tandem mass spectrometry. Levels were compared against established federal limits and by production method. Current-use pesticides and antibiotics were detected in several conventional (26–60 %; *n* 35) but not in organic (*n* 34) samples. Among the conventional samples, residue levels exceeded federal limits for amoxicillin in one sample (3 %) and in multiple samples for sulfamethazine (37 %) and sulfathiazole (26 %). Median bGH and IGF-1 concentrations in conventional milk were 9·8 and 3·5 ng/ml, respectively, twenty and three times that in organic samples ($P < 0{\cdot}0001$).

Introduction: Pesticides are widely used in U.S. food production, but the extent to which consumers are exposed to pesticides in milk is unknown, as is whether exposure differs when the milk consumed is produced using organic vs. conventional methods.

Antibiotics are also commonly used in food production (Landers et al. 2012), but only one known study, done a decade ago, has compared antibiotic residues in organic vs. conventionally produced milk (Vicini et al. 2008).

Bovine growth hormone (bGH) has also not been tested in organic versus conventional milk.

Given the prominent role of milk in the U.S. diet, particularly in the diets of children and those whose consumption continues into adulthood, this study was done to assess the extent to which pesticides, antibiotics, and synthetic hormones are present in retail milk and to determine how levels compare when produced using organic vs. conventional methods.

Material and methods: Milk samples were collected nine regions dividing the continental United States. Milk samples were spiked with isotopically labeled internal standards, when available, subjected to liquid-liquid or solid phase extraction, and pre-concentrated. Samples were tested for the residues of commonly used pesticides and antibiotics, as determined in consultation with dairy industry experts, using previously validated methods. Pesticide analysis was conducted via gas chromatography-tandem mass spectrometry with stable isotope (^{13}C) dilution quantification. The LODs for each analyte varied but were in the low pg/mL, ranging from 0.02 pg/mL for atrazine to 20 pg/mL for chlorpyrifos and cypermethrin. Analysis for antibiotic residues was performed via high performance liquid chromatography-high resolution linear ion trap where the LTQ accumulates, isolates, and fragments ions for MS/MS confirmation. Isotopically labeled internal standards were not available so external calibration was performed for quantification. Standards were purchased from Sigma-Aldrich (St. Louis, MO). The LOD for antibiotics was 1 ng/mL. Hormones were measured using a slight modification of the method of Kay et al. (2009) to allow for milk analysis using ultraperformance Liquid-Chromatography (UPLC)-high resolution mass spectrometry using a Velos LTQ Orbi-trap. The LODs for bGH and IGF-1 were 0.40ng/mL and 0.10 ng/mL, respectively. Similar to antibiotics, external calibration without stable isotopes was used for quantification. All studies included a concurrent analysis of positive and negative controls (10%) to ensure method validity. Positive controls consisted of pooled organic cows' milk that was spiked with the target compounds at a concentration that was 10x the method LODs. These milk samples also contained residual levels of the target chemicals so could not be used alone for method blanks.

The proportions of milk samples with detectable levels of each of the pesticides and antibiotics were calculated and Fisher's exact test of proportions were used to compare them by milk type (organic vs. conventional). The pesticide levels in each sample were also compared to existing federal tolerance limits. Due to deviations from a normal distribution, median levels of all chemicals of interest were reported for each milk type. Samples with values below the LOD were imputed as one-half the LOD of the laboratory method used for each chemical. Comparisons between median values of organic vs. conventional milk were done using Wilcoxon Rank-Sum tests. To facilitate comparison with an earlier study, the analysis for antibiotic and growth hormones was repeated to obtain adjusted least-squares means controlling for % milk fat and region using generalized linear models (GLM). All statistical analyses were performed using SAS version 9.4, and a two-sided *P* value of < 0.05 was considered statistically significant.

Results: *Pesticide results*

Residues of several currently used pesticides, including atrazine, chlorpyrifos, cypermethrin, diazinon, hexachlorobenzene, and permethrin were detected in many of the conventional milk samples (26%>60%) but in none of the organic samples (Table 2). Pesticide levels in the conventional samples were below the FDA limit for all but one of the pesticides (Table 2). Chlorpyrifos levels exceeded the limit of 250 pg/mL in four (11%) of the 35 samples (298, 304, 314, and 319 pg/mL). All samples (conventional and organic) were free of detectable levels of the pesticides dicofol, endosulfan-alpha, chlorthalonil, fonofos, cyfluthrin, and fenvalerate. Legacy pesticides, those now prohibited but that remain environmentally persistent, hexachlorobenzene and ppDDT, and the DDT metabolite/degradant, ppDDE, were detected in nearly all of the organic as well as the conventional samples (91%>100%; Table 2). ppDDT was the only pesticide to have a median level that was not statistically significantly higher in conventional compared to organic samples ($p = 0.38$).

Antibiotic results

The number of organic and conventional milk samples with detectable levels of antibiotic residues are presented in Table 1. While residues of at least one antibiotic were found in most of the conventional milk samples (60%), none were

detected in any of the organic samples. The median and ranges for all antibiotics tested are reported in Table 2. Estimated median levels of amoxicillin, oxytetracycline, sulfamethazine, sulfamethoxydin, and sulfathiazole were all statistically significantly higher in conventional compared to organic milk (p = <0.0001 - 0.0018). One of the 35 conventional samples (3%) had an amoxicillin residue level of 10.2 ng/mL, exceeding the FDA limit of 10.0 ng/mL. None of the samples had detectable levels of trimethoprim, monensin, or iasalocid.

Hormone results

Median levels in conventional milk samples were 9.8 ng/mL for bGH and 3.5 ng/mL for IGF-1, approximately 20 and 3 times higher (p < 0.0001), respectively, than the 0.5 ng/mL and the 1.1 ng/mL in the organic samples. Results of the sensitivity analysis adjusting for percent milk fat and region were similar to those obtained in the primary analysis. The adjusted least-squares mean (95% CI) for bGH was 9.4 (8.2-10.7) ng/mL and for IGF-1 it was 3.9 (3.1-4.6) ng/mL and both still differed significantly from their organic counterparts (p < 0.0001).

Discussion: Residues of current-use pesticides and antibiotics appear to be common in conventional but not organic milk sold through retails stores across the United States, at times exceeding federal tolerance limits. Similarly, growth hormone and IGF-1 levels were several times higher in conventional milk, which suggests that the difference reflects the use of synthetic growth hormones. While further research is needed to understand the lifetime risk, if any, to milk consumers resulting from their exposure to these chemicals, the findings of this study suggest that choosing to consume milk produced organically would minimize exposure and any possible associated risks.

References: Landers TF, Cohen B, Wittum TE *et al.* (2012) A review of antibiotic use in food animals: perspective, policy, and potential. *Public health reports (Washington, DC : 1974)* 127, 4-22.

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Image:

Table 1. Percent of Retail Milk Samples with Detectable Levels of Pesticide and Antibiotic Residues by Production Method (Conventional vs. Organic)

	Conventional (n=35)	Organic (n=34)	
Pesticides[1]	N (%) Detected	N (%) Detected	P-value[1]
Hexachlorobenzene	35 (100%)	34 (100%)	-
ppDDE	35 (100%)	34 (100%)	-
ppDDT	32 (91%)	32 (94%)	1.00
Atrazine	9 (26%)	0	**0.002**
Diazinon	21 (60%)	0	**<0.0001**
Chlorpyrifos	20 (59%)	0	**<0.0001**
Cypermethrin	17 (49%)	0	**<0.0001**
Permethrin	16 (46%)	0	**<0.0001**
Dicofol	0	0	-
Endosulfan-alpha	0	0	-
Chlorthalonil	0	0	-
Fonofos	0	0	-
Cyfluthrin	0	0	-
Fenvalerate	0	0	-
Antibiotics	N (%) Detected	N (%) Detected	P-value
Penicillins			
Carbenicillin	0	0	-
Amoxicillin	15 (43%)	0	**<0.0001**
Tetracyclines			
Oxytetracycline	21 (60%)	0	**<0.0001**
Sulfonamides			
Sulfamethazine	13 (37%)	0	**0.0001**
Sulfabromethazine	0	0	-
Sulfadimethoxydine	18 (51%)	0	**<0.0001**
Sulfapyridine	0	0	-
Sulfathiazole	9 (26%)	0	**0.002**
Ionophores			
Monensin	0	0	-
Lasalocid	0	0	-
Pyrimidine inhibitor			
Trimethoprim	0	0	-

[1]P-value for Conventional vs. Organic: Fisher's exact test of proportions.

Image 2:

Table 2. Median and Ranges for Pesticides and Antibiotic Residues in Retail Milk Samples (Conventional vs. Organic)

		Conventional (n=35)	Organic (n=34)	
Pesticides	**EPA Limit for milk (pg/mL)**	**Median (range) pg/mL**	**Median (range) pg/mL**	**P-value[1]**
Atrazine*	20	<0.01 (<0.01-0.29)	<0.01	**0.0018**
Hexachlorobenzene	NA	236 (49.0-553)	45.5 (23.0-225)	**<0.0001**
ppDDE	NA	465 (206-694)	303 (110-492)	**<0.0001**
ppDDT	NA	51.6 (<5-137)	45 (<5-131)	0.38
Diazinon*	NA	29.0 (<5-150)	<5	**<0.0001**
Chlorpyrifos*	250	**112 (<20-319)**	<20	**<0.0001**
Cypermethrin*	2500	<20 (<20-210)	<20	**<0.0001**
Permethrin*	3000	<5 (<5-184)	<5	**<0.0001**
Dicofol	-	<5	<5	-
Endosulfan-alpha	-	<5	<5	-
Chlorthalonil	-	<5	<5	-
Fonofos	-	<5	<5	-
Cyfluthrin	-	<5	<5	-
Fenvalerate	-	<5	<5	-
Antibiotics	**FDA Limit (ng/mL)**	**Median (range) ng/mL**	**Median (range) ng/mL**	
Penicillins				
Carbenicillin	-	<1	<1	-
Amoxicillin*	10	**<1 (<1-10.2)**	<1	**<0.0001**
Tetracyclines				
Oxytetracycline*	300	14.6 (<5-147.2)	<5	**<0.0001**
Sulfonamides				
Sulfamethazine*	10	<1 (<1-6.8)	<1	**<0.0001**
Sulfabromethazine	-	<1	<1	-
Sulfadimethoxydine*	10	1.2 (<1-7.2)	<1	**<0.0001**
Sulfapyridine	10	<1	<1	-
Sulfathioazole*	10	<1 (<1-6.0)	<1	**0.002**
Ionophores				
Monensin	-	<10	<10	-
Lasalocid	-	<10	<10	-
Pyrimidine inhibitor				
Trimethoprim	-	<1	<1	-

[1] P-value for Conventional vs. Organic: Wilcoxon Rank-Sum test

Note: Wilcoxon-rank sum tests for differences in population mean ranks (distributions) and is not a median test (note significantly different samples with the same median values)

Samples with values <LOD are indicated with "<Y" where Y is equal to the limit of detection for that chemical. Estimated values for samples <LOD were imputed at ½*LOD for purposes of testing the differences between groups.

*Percent of total sample below the LOD is >50%; refer to Table 2 for comparisons of conventional vs. organic samples using proportions detected instead (recommended by Helsel et al.).

Results presented in bold indicate those antibiotics and pesticides for which the residue levels in at least one milk sample exceeded federal limits.

Disclosure of Interest: None Declared

Keywords: antibiotic residues, dairy, growth hormones, milk, pesticide residues