Trace Elements and Organochlorine Pesticides in Raw Milk from South Eastern Regions of Romania

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Received: August 9, 2011 / Published: March 20, 2012.

Abstract: A total of 108 raw milk samples were collected from tankers arriving at processing facilities from rural areas in eastern and northern regions of Romania from January to December 2010. Concentrations of As, Cu, Zn and Pb in the samples were performed using an atomic absorption spectrophotometer. Detection and measurement of the organochlorine pesticides residues ($\alpha + \beta$ -HCH, γ -HCH and total DDT) were achieved by using a gas chromatograph equipped with a ^{63}Ni electron capture detector. Pb contents in all samples corresponded to tolerance level (0.1 mg kg^{-1}) and in 98% of these samples were below 0.05 mg kg^{-1} . All samples examined for residues of Cu, Zn and As were completely acceptable as well. Residue levels of organochlorine pesticides in all analysed samples were far below tolerance levels, too. In 93.5% of samples $\alpha + \beta$ -HCH and in 85% of samples γ -HCH was below the limit of detection (0.008 mg kg^{-1}). In 85% of samples total DDT residues were between < 0.005 and $0.0068 \text{ mg kg}^{-1}$. In future studies, a greater number of milk samples from different regions of Romania should be controlled to confirm the absence of possible toxicological risks.

Key words: Cow milk, trace elements, organochlorine pesticides, atomic absorption spectroscopy, Romania.

1. Introduction

Milk is considered as a nearly complete food since it is a good source for protein, fat and major minerals. Also, milk and milk products are main constituents of the daily diet, especially for vulnerable groups such as infants, school age children and old age [1].

However, milk and dairy products may contain varying amounts of different toxic contaminants [2-4]. As an excretion of the mammary gland, it can carry numerous xenobiotic substances (pesticides, disinfectants, drugs, metals and various environmental contaminants), which constitute a technological risk factor for dairy products, for the related commercial image and, above all, for the health of the consumer.

Today, the consumer is more demanding than in the past and expects "healthy" milk, rich in nutrients, with high biological value, but without health risks [5].

Trace elements are fairly widespread in the environment. Their level with an anthropogenic origin is much higher than the level from natural sources. These elements are non-essential for almost all living organisms. The largest amount of trace elements found in humans has been absorbed through food. Organochlorine pesticides are very toxic organic compounds of anthropogenic origin which pose a serious threat to the environment and human health.

The main organochlorine pesticides are considered to be persistent organic pollutants. These compounds are a special problem because they persist in the environment for a long time before they break down, travel over long distances to all parts of the globe and poison humans and wildlife. They tend to concentrate

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in the fatty tissues of humans and animals that are at the top in the food chain. The only way that the organisms can excrete them is through milk [6].

Many countries have adopted regulations that state the maximum residue levels of environmental pollutants in food in order to protect consumers. Current maximum residue levels for some environmental pollutants in cow's milk in Romania according with EU regulation are presented in Table 1.

2. Material and Methods

A total of 108 raw milk samples (330 mL) were collected from tankers arriving at processing facilities from rural areas from eastern and northern counties of Galati during January until December 2010. Upon collection, all the samples were placed in sterile bottles, stored at 4-6 °C in a cool box and immediately transported to the laboratory and stored at -20 °C until analysis.

2.1 Analysis of Toxic and Trace Elements

The dosage of metallic ions in milk was accomplished with the help spectrometry of atomic absorption using the Avanta PM. GBS Scientifique Equipment Victoria (Australia). For the other metals the decomposition by wet way was used according to the AOAC methods [9]. From the final solution there are determined through chemical methods, according to the national standards: copper, lead, zinc and arsenic. For the spectrometry of atomic absorption the hydrochloric solutions are preferred, because the metals halogenure are volatile in flame. Both for the verification of the reproducibility of the results, and for the better conservation of metals present in the traces, the milk samples were decomposed by wet way according to the national standard 8342/89, by incineration and calcinations at 500 °C, is finally dissolved in HCl 5 N. The AAS apparatus functions with air-acetylene flame at a flame rate of 10 L min⁻¹ air and 0.99 L min⁻¹ acetylene in calibrating conditions for $R^2 = 0.994$ [10].

Table 1 Maximum residue levels for some trace elements and organochlorine pesticides in cow's milk in Romania (all values in mg/kg according to the Regulation EC No 1881/2006 [7] and Regulation (EU) No 600/2010 [8]).

Trace elements		Organochlorine pesticides	
Pb	0.05	Total DDT	0.04
Cu	0.5	α -HCH	0.004
As	0.05	β -HCH	0.003
Zn	5	γ -HCH	0.001

Analyses of Pb, Cu, As, Zn and organochlorine pesticides have been carried out at the National Sanitary Veterinary and Food Safety Authority in Galati, Romania. This paper presents the results of residue monitoring and environmental pollutant scanning of Romanian raw cow's milk from January to December 2010.

2.2 Sample Preparation, Extraction and Cleanup

Fat were extracted from milk according to the method of the AOAC [11]. Briefly three grams or less of the fat was dissolved into 40 mL petroleum ether. This was partitioned three times into acetonitrile saturated with petroleum ether (3 × 30 mL). The acetonitrile fraction, after dilution with saline (600 mL), was again partitioned into petroleum ether (3 × 100 mL). This was dried over anhydrous sodium sulfate, and concentrated at 30 °C on a rotary vacuum evaporator to a volume less than 5 mL to be used for Florisil cleanup [12].

Cleanup of the extracted samples, to remove the residual fat, was performed by transferring the extract into a glass chromatographic column (25 mm internal diameter) containing 25 g activated Florisil (60/100 mesh) topped with 1-cm layer of anhydrous sodium sulfate.

The prepared column was rinsed with 100 mL petroleum ether, and then the extracted sample was transferred onto the column. The column was eluted with 300 mL eluent (20% dichloromethane + 80% petroleum ether). The collected eluate was concentrated to dryness on a rotary vacuum evaporator and dissolved in hexane to a volume of 5 mL [13]. An aliquot of each extract was transferred to 2 mL injection vials to be ready for the analysis with the electron capture gas chromatography.

2.3 Determination of Organochlorine Pesticide Residual Concentrations

The organochlorine pesticide residues were determined by analysis of samples using a Hewlett-Packard gas chromatograph (GC-HP 5890 Series II) equipped with a ^{63}Ni electron capture detector, using a silica capillary column (HP-5 30 m $\times$ 0.32 mm internal diameter with 0.25 μm film thickness). The carrier gas was helium at a flow rate of 2 mL min $^{-1}$ through column and 30 mL min $^{-1}$ make up.

The gas chromatography oven temperature was initiated at 80 °C for 2.2 min, raised to 175 °C (at a rate of 30 °C min $^{-1}$), then raised to 225 °C (at a rate of 10 °C min $^{-1}$) and held for 2 min. Injection port temperature and detector temperature were maintained at 280 °C and 300 °C, respectively. The sample volume injected was 1 μL . Calibration standard curves were created and organochlorine pesticide residues were quantitatively determined by comparison of the retention time and peak heights/areas of the sample chromatogram with those of standard solutions run under the same operating conditions. The concentrations of various residues in each sample were reported as mg kg $^{-1}$ on a fat basis [14].

2.4 Statistical Analysis

The data were analysed using Analysis of Variance (ANOVA). Comparison of means was done using Duncan's Multiple Range test at $P < 0.05$.

3. Results and Discussion

3.1 Milk Composition

Milk samples of different seasons were varied in their composition. These variations are primarily due to milk changes in fat, protein, lactose and occasionally the mineral salts content of milk (Table 2).

During the summer season, more fat and protein were found in milk samples than in winter season. The differences in fat and protein contents among the season were highly significant ($P < 0.05$). The lactose

content of milk samples of different season did not significantly vary.

This was expected since lactose plays an important role in maintaining the osmotic pressure of milk.

3.2 Milk level of Toxic and Trace Elements

The concentrations (mg kg $^{-1}$) of the trace elements (Cu and Zn) and the toxic metals (Pb and As) in the bovine milk samples from Galati are reported in Table 3.

This study demonstrates that, in milk from Galati County the concentrations of toxic metals (As and Pb) were below detection limits. In the present study, As concentrations ranged from minimum values of 0.001 mg kg $^{-1}$ in the north and 0.005 mg kg $^{-1}$ in the east region and there were no significant differences in arsenic levels between the regions. Significant differences in arsenic levels between traffic and industrial regions (0.05 and 0.04 mg kg $^{-1}$) and rural regions (0.0002 mg kg $^{-1}$) were reported in Turkey [15].

Pb in all samples studied albeit not in dangerous concentrations, showing how this metal is ever more frequently found in milk. The presence of Pb in milk samples could be due to various factors: transhumance along roads and/or motorways, fodder contamination, climatic factors, such as winds, and the use of pesticide compounds [5, 16].

Therefore, it is necessary to monitor this metal over time to better clarify its presence in milk. The average lead content of our milk samples was between 0.001-0.005 mg kg $^{-1}$.

Higher lead levels in milk were reported in an unpolluted area (0.25 mg L $^{-1}$) and around plant and smelter areas in India (0.65-0.85 mg L $^{-1}$) [17], and also in Brazil (0.23 mg L $^{-1}$) [18] and Pakistan (21.78 and 15.96 mg L $^{-1}$) [19].

The levels of trace elements (Cu and Zn) found were below detection limits. Copper is an essential element required in the diet due to its role in vital oxidation-reduction reactions. At supraoptimal concentrations, copper may generate toxic effects such as, dermatitis, liver cirrhosis and neurological disorders

Table 2 Mean composition of cow's milk samples collected from different locations of Galati County in 2010.

Parameter/Component	Locations		
	Schela	Brănești	Barboși
Density (g/cm ³)	1.0285 ± 0.0008 ^a	1.0269 ± 0.0011	1.0262 ± 0.0004
Acidity (°T)	18.75 ± 0.5762	18.58 ± 0.5293	18.25 ± 0.4154
Total dry substance (%)	12.06 ± 0.2881	11.67 ± 0.2881	11.23 ± 0.2412
Fat (%)	3.64 ± 0.1742	3.64 ± 0.1876	3.44 ± 0.2077
Protein (%)	3.36 ± 0.0804	3.20 ± 0.0804	3.11 ± 0.0402
Lactose (%)	4.38 ± 0.0938	4.19 ± 0.1072	4.06 ± 0.0402
Mineral salts (%)	0.68 ± 0.0201	0.64 ± 0.0201	0.62 ± 0.0067
Freezing point (°C)	-0.515 ± 0.0181	-0.493 ± 0.0181	-0.469 ± 0.0067

^a: values ± standard errors of regression.

Table 3 Trace elements and toxic metals in milk.

Locations	Trace elements		Toxic metals	
	Cu	Zn	Pb	As
Schela	0.29 ± 0.039* (0.22 ÷ 0.35)	3.38 ± 0.073* (3.26 ÷ 3.47)	0.0023 ± 0.00089* (0.001 ÷ 0.003)	0.0013 ± 0.00045* (0.001 ÷ 0.002)
Brănești	0.33 ± 0.064* (0.22 ÷ 0.43)	3.37 ± 0.069* (3.28 ÷ 3.5)	0.0026 ± 0.0009* (0.001 ÷ 0.004)	0.0018 ± 0.00083* (0.001 ÷ 0.003)
Barboși	0.28 ± 0.043* (0.23 ÷ 0.37)	3.35 ± 0.073* (3.21 ÷ 3.45)	0.0038 ± 0.00075* (0.003 ÷ 0.005)	0.0027 ± 0.0014* (0.001 ÷ 0.005)

The values were expressed in mean ± standard errors of regression and values in parenthesis indicate minimum and maximum level recorded. The mean value with similar superscripts not varied significantly at $P < 0.05$.

[20]. The low concentrations of Cu could be due to Zn contained in food that interferes with the copper absorption system, explaining the presence of low levels of this metal in milk.

Zinc concentrations were measured in all raw milk samples and were between 3.26-3.5 mg kg⁻¹. These values are similar to those found in other studies (21 to 41 μg g⁻¹; 20 to 34 μg g⁻¹) [21]. The copper levels were higher than those reported in southern Poland (0.2-0.3 mg kg⁻¹) [22], Spain (60 μg g⁻¹ and 51.8 μg L⁻¹) [23], Calabria in Italy (0.14-737.58 μg kg⁻¹) [5] and unpolluted areas of India (0.101 mg L⁻¹) [16].

3.3 Organochlorine Pesticide Residues in Milk

The present study showed the presence of organochlorine pesticide residues in milk owing to their use in sanitary and agricultural purposes.

Residues of α + β-HCH (Fig. 1) and γ-HCH (Fig. 2) in all 108 analyzed samples of raw cow's milk were under the limit of detection (0.004 mg·kg⁻¹ and 0.0008 mg kg⁻¹). In 93.5% of samples α + β-HCH and in 85% of samples γ-HCH was below the limit of detection.

The present results revealed that the frequency of different isomers of HCH residues in dairy products on fat basis was of the order of $\gamma > \alpha + \beta$. In addition, with regard to mean concentration in the contaminated samples γ-isomer was highest (0.0026 mg kg⁻¹ fat, location 3-Barboși) followed by α + β-isomer (0.0004 mg kg⁻¹ fat location 3-Barboși).

The ratios and levels of HCH isomers are often used as evidence of the passed or current technical HCH application [24].

In all analyzed samples of milk, collected from Galati County, total DDT levels (Fig. 3) were below the limit of detection (0.04 mg kg⁻¹). In 85% of samples total DDT residues were between < 0.005 and 0.0068 mg kg⁻¹. The highest content of total DDT were 0.0068 mg kg⁻¹ for location 3 (Barboși) and the lowest content were 0.0016 mg kg⁻¹ for location 1 (Schela).

4. Conclusions

Residues of environmental pollutants examined in Romanian raw cow's milk were not problematic from January until December 2010; milk is safe for the

Fig. 1 Variation of the $\alpha + \beta$ -HCH content.

Fig. 2 Variation of the γ -HCH content.

Fig. 3 Variation of the total DDT content.

consumers. In conclusion, our data deserve particular attention not only for their significance but especially because they were recorded in Galati, a region with a high risk of environmental pollution due to the of industries. Further studies are necessary to evaluate the contents of trace elements, toxic metals and organochlorine pesticides on a greater number of milk samples from various dairy farms in Galati and to confirm the absence of possible toxicological risks in this region.

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