PHTHALATES IN COW MILK DEPENDING ON THE METHOD OF MILKING

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ABSTRACT

The content of phthalic acid esters (PAE) in samples of cow milk obtained by hand and machine milking using HPLC was studied. Five cows for hand milking and five cows for machine milking were included in the experiment. A mixed sample from the morning and evening milking was obtained from each cow. Sampling was performed for the period of five days. For samples of individual cows obtained by machine milking, average concentrations of di-n-butyl phthalate (DBP) ranged from 6.28 ± 2.82 $mg.kg^{-1}$ and 10.43 ± 3.59 $mg.kg^{-1}$ and average concentrations of di-(2-ethylhexyl) phthalate (DEHP) ranged from 0.05 ± 0.07 $mg.kg^{-1}$ and 0.20 ± 0.17 $mg.kg^{-1}$. For samples obtained from individual cows by hand milking in January, average concentrations of di-n-butyl phthalate (DBP) ranged from 2.76 ± 1.20 $mg.kg^{-1}$ to 7.02 ± 4.26 $mg.kg^{-1}$ and di-(2-ethylhexyl) phthalate (DEHP) from 0.01 ± 0.01 $mg.kg^{-1}$ and 0.06 ± 0.06 $mg.kg^{-1}$. Statistically strongly significantly lower ($p < 0.01$) average concentrations of DBP and DEHP were found in samples of milk obtained by hand milking. Statistically ($p < 0.01$) significant differences between the average concentrations of DBP and DEHP were demonstrated.

Key words: DBP, DEHP, phthalates, cow milk, milking

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INTRODUCTION

Phthalic acid esters (PAE - phthalic acid esters, phthalates) belongs to the group of virtually ubiquitous organic environmental contaminants (Velíšek, 2002). Environmental Protection Agency (EPA) in the USA has included six phthalic acid esters (PAE) of the priority hazardous pollutants. These include dimethyl-phthalate (DMP), diethyl-phthalate (DEP), di-n-butyl phthalate (DBP), di-2-ethylhexyl phthalate (DEHP), di-n-octyl phthalate (DOP) and dibutylbenzyl phthalate (BBP) (Jarošová, 2000). Phthalates of higher molecular weight, such as di-2-ethylhexyl phthalate (DEHP), are primarily used as plasticizers in polyvinyl chloride (PVC) products, while lower molecular weight phthalates, such as diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBzP) are widely used as solvents and fixation agents in perfumes and as an ingredient in personal care products and cosmetics (Cao, 2010). They are not chemically bound to the polymer, so they can be released into the environment, and therefore are found in all environmental media (Velíšek, 2002). Large amounts of phthalates, however, are not released only during their use, but also during the management of plastic waste (sending to landfill, incineration). This results in leakage of roughly 63 per cent. The mainly polluted element of the environment is soil (about 77 per cent) followed by water (21 per cent) (Hunter and Uchrin, 2000). The most abundantly occurring phthalate in the environment is DEHP (Latini, 2005).

Although the acute toxicity PAE is relatively low, continuously running production exposes the population to chronic exposure. Long-term effects on living organisms are the subject of research in many laboratories (Jarošová, 2000). Chronic revenue of phthalates, especially of di-2-ethylhexyl phthalate, may also have teratogenic and carcinogenic effects (liver cancer cell) and affect the reproductive ability of the body (decreased weight of the testes, ovaries, sperm count) (Velíšek, 2002). Phthalates potentially disrupt the human hormonal system, sexual development, reproduction and potentially encourage asthma and skin diseases of young children (Wormuth et al., 2006). Some phthalates are considered developmentally toxic substances harmful to reproduction (Witassek et al., 2011).

Humans can be exposed to phthalates after ingestion of food or water (orally), from the air (inhalation), through dermal absorption or parenteral application (Cory-Slechta, 2008). The most important from these options is the intake through food, in particular through those foodstuffs that have a high fat content which accumulates phthalates (Velíšek, 2002). Due to their lipophilic nature phthalates may also lead to accumulation of the feed and the environment in animal tissues, muscle, fat, and also may phthalates pass from the digestive tract to the milk, which leads to another potential threat chain and of the person (Rhind et al., 2005).

The aim of this study was to investigate phthalic acid esters in samples of cow's milk obtained by hand and machine milking.

MATERIAL AND METHODS

Chemicals

Analytical standards of DBP and DEHP from the company of Supelco (USA) with a minimum purity of 99.9 per cent were used for the analytical determination of phthalates. Basic and working solutions were diluted with acetonitrile with HPLC purity for residues. The solvents n-hexane, cyclohexane, dichloromethane and acetone with purity for residues were applied. Sulfuric acid was of analytical grade. Water was deionized and purified using Mili-Q-patron.

Milk Samples

Cow milk samples were collected from a farm located in the Southmoravian Region where both hand and machine milking were possible. Samples of 250 ml were collected at the farm in glass containers with lids with polytetrafluorethylene (PTFE) sealings. Five cows for hand milking and five cows for machine milking were included in the experiment. A mixed sample from the morning and evening milking was obtain from each cow. This sample was cooled down and subsequently frozen. Sampling was performed for the period of five days.

Methods Applied

To detect the PAE, proven methods for determining DBP and DEHP in foodstuffs were utilized (Jarošová at al., 1998, 1999).

Milk samples were homogenized, weighed (400-600 g) into metal bowls and frozen. Gradually, frozen samples were lyophilized and subsequently PAE residues were extracted using n-hexane. PAE were separated from co-extracts by gel permeation chromatography employing the gel of Bio beads S-X3. The cleaning procedure with concentrated sulfuric acid was used for final purification of the eluate. Determination of PAE was performed by high performance liquid chromatography (HPLC), liquid chromatograph of Agilent Technologies LC/MSD VL by column of Zorbax Eclipse XDB-C8, 150 x 4.6 mm, 5 µm grain, mobile phase acetonitrile:water changed elution with time as follows: 0 to 3 min – 80:20, 3 to 9 min – 95:5, 9 to 12 minutes – 100:0, 13 to 18 min – 80:20. Evaluation was performed utilizing the Agilent chemstation software.

All laboratory glass was rinsed by hexane during sample preparation. Simultaneously, dry matter and fat content were determined for each sample. All samples were analyzed in duplicate. Concentrations of DEHP and DBP are related to the original sample.

The results were statistically processed in Microsoft Excel and STATISTICA 10. Duncan's test was applied.

RESULT AND DISCUSSION

Average concentrations of DBP and DEHP in all milk samples collected in January 2013 by hand and machine milking are presented in Figure 1. Each value represents the average of the five values (n=5, the number of cows).

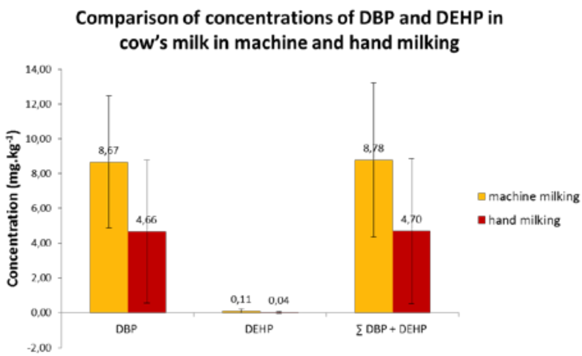

Fig. 1 *Average concentrations of DBP and DEHP ($\bar{x}$ ± S.D) mg.kg^{-1} in the original sample in samples of cow's milk collected by hand and machine milking*

Average concentrations of DBP for samples of individual cows obtained by machine milking ranged from 6.28 ± 2.82 mg.kg^{-1} and 10.43 ± 3.59 mg.kg^{-1} and DEHP from 0.05 ± 0.07 mg. kg^{-1} to 0.20 ± 0.17 mg.kg^{-1}. For samples of individual cows obtained by hand milking, average concentrations of DBP ranged from 2.76 ± 1.20 $mg.kg^{-1}$ and 7.02 ± 4.26 $mg.kg^{-1}$ and DEHP from 0.01 ± 0.01 mg. kg^{-1} to 0.06 ± 0.06 $mg.kg^{-1}$.

Statistically strongly significantly lower ($p < 0.01$) average concentrations of DBP and DEHP were found in samples of milk obtained by hand milking.

Statistically highly significant difference ($p < 0.01$) between average concentrations of DBP or DEHP for individual cows in a single method of milking was found.

Yong-Lai et al. (2005) found that phthalic acid esters released from PVC hoses used for milking could be a source of potential contamination of milk and milk products. As in the study by Fierens et al. (2012) a lower average concentration of contamination of milk samples milked by hand (100 $\mu g.kg^{-1}$ fat) compared to samples milked by a machine (179 $\mu g.kg^{-1}$ fat) were revealed, it was concluded that milking devices are an important source of milk contamination. These findings can support the results obtained in our experiment, since statistically strongly significantly lower ($p < 0.01$) average concentrations of DBP and DEHP were found in milk samples milked by hand.

Differences in accumulation of DEHP and DBP in cow milk may be caused by different metabolism of cows, ratio between accumulation and elimination of phthalates from the body and partially by different physico-chemical properties of both phthalates. DBP has a smaller molecule with a shorter unbranched chain allowing partial solubility of DBP in water. DEHP is insoluble in water.

Rhind et al. (2005) reported that, due to the lipophilic nature of phthalates, their accumulation from the feed and environment in animal tissues, muscle, fat can occur and also phthalates may pass from the digestive tract to the milk, which leads to another potential threat of the food chain and thereby to humans.

CONCLUSIONS

Currently effective legislation in the Czech Republic does not cover the issue of phthalates in foodstuffs. Under the Act No. 110/1997 Coll., on Foodstuffs and Tobacco Products, and Decree No. 53/2002 Coll., phthalates content was regulated by determining the permissible amount of the sum of DEHP and DBP. Allowable amounts of these phtalates in spirits were determined in the amount of 1 $mg.kg^{-1}$, in child and infant nutrition, and in the so-called basic foodstuffs, in muscle of livestock in the amount of 2 $mg.kg^{-1}$ of the original sample and in fat 4 $mg.kg^{-1}$. However, by adoption of EU legislation in 2004 these limits were omitted from the Decree. Nevertheless, assuming the validity of these limits, all analyzed milk samples regardless of the date and type of milking were found to be unsuitable.

Therefore, these compounds ought to be monitored. Necessity of potential provisions on the legislative limits for phthalates in feed should be considered in order to prevent potential contamination of the food chain.

One way to gradually reduce the risks of phthalates is to promote the substitution of toxic phthalates by other health-harmless substances such as citrates, phenol alkyl sulfonates, benzoates, especially in the production of materials used in agriculture, food industry, and health care.

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