RISK ASSESSMENT PRACTICE IN HYGIENIC AND EPIDEMIOLOGICAL STUDIES

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ASSESSMENT OF HEALTH RISK CAUSED BY PHTHALATES PENETRATING A BODY WITH MILK IN POLYMER AND POLYMER-CONTAINING PACKAGE

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Since the middle of the 20th century phthalates have been widely used in food products package manufacturing. But here phthalates turned out to migrate from this package into the environment. There are some data on unfavorable impacts exerted by orally introduced phthalates on the liver and hormonal system.

Milk packed in polymer package which contains various plasticizers including phthalates is widely spread on the Russian consumer market. It determined our research goal which was to assess consumers health risks related to impacts exerted by phthalates introduced with milk packed in polymer package.

We selected 25 milk samples out of milk products bought in retail networks. Phthalates were quantitatively determined in milk via highly efficient liquid chromatography. We performed a distribution questioning to assess quantity and volumes in which milk was consumed and to determine a share of milk packed in polymer package. We detected that 57 % adult respondents, 75 % children aged 4–6, and 80 % children aged 7–17 consumed milk packed in polyethylene film and (or) in plastic bottles. 5 phthalate forms were identified in consumed milk. Adults actually consumed 0.6 liter of milk per day; children aged 4–6, 0.2 liter; children aged 7–17, 0.3 liter. Phthalates dose introduced daily with milk was equal to $5.61 \cdot 10^{-2}$ mg/kg of body weight for children aged 4–6; $6.32 \cdot 10^{-2}$ mg/kg of body weight for children aged 7–17; $4.20 \cdot 10^{-2}$ mg/kg of body weight for adults.

We calculated a lifelong risk and revealed that it occurred due to di-2-ethylexylphthalate when milk packed in polyethylene film and plastic bottles was consumed. Risk-characterizing hazard indexes reached 1.85 for the liver and endocrine system regardless of package.

Key words: *phthalates, milk, plasticizers, polymer package, package, dose, exposure, risk assessment, questioning.*

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Phthalates (phthalic acid ethers) were first synthesized in 1920s. In 1930s manufacturers started to actively apply them to produce plastics as phthalates made these materials more durable and flexible. Since 1950s phthalates have been widely used in many industries, not only as plasticizers, but also as additives into cosmetics, food products, etc. [1].

Influence exerted by phthalates on consumers' health has been concurrently examined. There are data on contribution made by phthalates into oxidative stress evolvement, disorders in substances transport and gall excretion [2], malignant neoplasms in the liver [3] and the pancreas [4] in rats. Some research performed in various countries over the last few decades contain data on a determined direct correlation between phthalates occurrence in a body and reproductive disorders [5–7], or evidence that disorders in fetus' gonads development are quite possible [8]. A reverse correlation has been established between mono-n-butyl phthalate (MBP) contents in urine and free thyroxin (FT4) level and thyroxin (T4) level, and an assumption has been made that di-n-butyl phthalate (DBP) can violate thyroid gland activity in pregnant women [9]. The obtained data allowed to consider phthalates to be endocrine disruptors, or substances which can disrupt hormones' normal functioning.

By the end of 2014 the following phthalates were most widely spread in the industry worldwide: di-butyl-phthalate (DBP), butylbenzyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP), diisononyl phthalate (DINP), diisodexyl phthalate (DIDP), dioctyl phthalate (DOP), diethyl phthalate (DEP), dimethyl phthalate (DMP). DEP, DMP, DBP, BBP, and DEHP are the most dangerous among them; these phthalates are assigned into short-chain phthalates group according to REACH (Registration, Evaluation, Authorization and Restriction of Chemical substances) classification which is a part of the EU legislation; short-chain phthalates are considered to be the most hazardous for human health [10].

Phthalates are not bound to polymers molecules in plastics structures. They can migrate into the environment under external factors influence or regardless of it. It is the most interesting research task to analyze phthalates migration out of polymer package into food products. Polymer package is a basic source of phthalates introduction into a human body [11–13].

The Customs Union Technical Regulation 005/2011 "On package safety" sets forth maximum specific migration limit (SML) for phthalates in the Russian Federation and the Eurasian Economic Union member countries. According to this document, SML is fixed only for dioctyl phthalate and is equal to 2 mg/l. Dibutyl phthalate migration and, consequently, occurrence is not permissible at all (sensitivity limit is not fixed)[1]. And there are no existing standards for other phthalates.

As milk is usually kept and sold in polymer package (polyethylene film, plastic and polypropylene bottles), it is interesting to examine phthalates concentrations in milk as well as health risk caused by consuming milk with phthalates. Phthalates dose here depends both on the level of their migration into a product, and on milk consumption volumes.

Figures obtained in the Russian Feder-

[1] TR 005/2011. On package safety: The Customs Union Technical Regulation 005/2011. Available at: http://www.eurotest.ru/upload/iblock/6c9/6c977dbc8c9f2fc095035f49b52985f1.pdf (15.11.2017).

ation in 2013 showed that about 65% men and 75% women aged 14 and older, as well as about 80% children aged 3-13 consumed milk and dairy products daily or several times a week. Annual average milk and dairy product consumption amounted to 305.4 kg for men (14 and older); women, 276.5 kg; children aged 3-13, 296.9 kg. As for Perm region, about 70% men and 75% women aged 14 and older, as well as about 85% children aged 3-13 consumed milk and dairy products daily or several times a week. Average annual milk and dairy products consumption amounted to 278.5 kg for men and 249 kg for women [13]. According to the RF Public Healthcare Ministry recommendations, daily milk and dairy products consumption should amount to 352 kg per capita annually, and milk consumption should be equal to 50 kg per year which corresponds to a daily standard being equal to 180 ml[2]. Perm region is at the 37th place among all the Russian regions in terms of milk and dairy products consumption per capita.

People most frequently consume milk which is sold in polymer package; it is either polymer film made of low density polyethylene (LDPE); or bottles made of polyethylene terephthalate (PET) and polypropylene (PP). All the above mentioned materials contain various plasticizers to some extent, including phthalates as well [14, 15].

Our research goal was to assess health risk related to impacts exerted by phthalates which entered a consumer's body with milk kept in polymer package. Our research tasks included determination of phthalates concentrations in milk and risk and exposure assessment.

Data and methods. To perform our research, we selected 24 milk samples with fat content being equal to 2.5-3.2%; milk was kept in polymer package (19 samples were in polyethylene film, 5 were in PET bottles). We analyzed such plasticizers in milk as dimethyl phthalate (DMP), dibutyl phthalate (DBP), di -ethylhexyl phthalate (DEHP), and butylbenzyl phthalate (BBP) via high performance liquid chromatography on Agilent 1200 liquid chromatograph with a diode matrix detector in conformity with Methodical guidelines[3]. We extracted phthalates from milk with solid-phase extraction technique.

Exposure was assessed allowing for consumption of milk with the maximum calculated phthalates concentration in it. Phthalates doses which entered consumers' bodies were calculated as per Guide P 2.2.1.10.1920-04 [16]. Our calculation was based on standard values for exposure duration and averaging time for non-carcinogenic substances at oral introduction.

Lifelong average daily phthalates dose introduced with milk kept in polymer package was calculated for each detected phthalate as per the following formula:

$$\text{LADD} = \frac{(EDb \cdot ADDchb) + (EDc \cdot ADDchc) + (EDf \cdot ADDcha)}{AT},$$

[2] On approval of recommendations on rational food products consumption standards meeting contemporary healthy nutrition requirements: The Order issued by the RF Public Healthcare Ministry on August 19, 2016 N 614. Available at: http://www.garant.ru/hotlaw/federal/898204/ (15.11. 2017).

[3] MG 4.1.3160-14. Measuring phthalates mass concentrations (dimethyl phthalate, diethyl phthalate, dibutyl phthalate, benzylbutyl ohthalate, and di-ethylhexyl phthalate) in milk with high performance liquid chromatography: Methodical guidelines. Available at: http://files.stroyinf.ru/Data2/1/4293761/4293761690.htm (22.11.2017)

were *LADD* is lifelong average daily dose, mg/(kg day);

EDb is exposure duration for babies (aged 0 - <6 years);

EDc is exposure duration for children (aged 6 - <18 years);

EDa is exposure duration for adults (aged 18 and older);

ADDChb is chronic average daily dose for babies, mg/kg a day;

ADDChc is chronic average daily dose for children, mg/kg a day;

ADDCha is chronic average daily dose for adults, mg/kg a day;

AT is averaging time, number of years.

Results and discussion. At hazard identification stage we determined which organs and systems were critical for phthalates: they were the liver, the pancreas, the endocrine system, and also the overall systemic effects were detected.

When we assessed "exposure - effect" relationship, we determined reference doses at phthalates oral introduction. Thus, according to R 2.1.10.1920-04 "Guidelines on assessment of population health risk under exposure to chemicals which pollute environment" existing in the RF, reference dose under chronic oral introduction is fixed at 0.2 mg/kg of body weight for BBP (target organs are the liver and the pancreas); for DEHP, at 0.02 mg/kg of body weight (target organs are the liver and the endocrine system); for DBP, at 0.1 mg/kg of body weight (systemic effects): for DMP, at 10 mg/kg of body weight (the target organs are the kidneys); for DEP, at 0.8 mg/kg of body weight (systemic effect) [16].

As we assessed exposure, we detected that 58% milk samples in polyethylene film, and 40% milk samples in PET bottles contained phthalates (Table 1). DEHP made the greatest contribution into the total phthalates concentrations in milk in polyethylene film (70%), and DBP, in milk in PET bottles (65%).

At this stage we detected maximum phthalates doses for each group which entered consumers' bodies with milk in polymer package (Table 2). We allowed for body weight of people from the examined groups in our calculations.

According to the questioning results 75% children aged 4-6 consumed milk. Their parents bought milk only in polyethylene film. Children daily consumed 0.1-0.2 l of milk.

We revealed that 80% examined school children aged 7-17 consumed milk. Their parents bought milk in polyethylene film (in 68% cases), in PET bottles (in 16% cases), or in both packages by turns (in 16% cases). Daily milk consumption in this group amounted to 0-0.3 l. 57% of all the questioned adults consumed milk and they all chose milk in polyethylene film. Adults daily consumed about 0.1-0.6 l of milk.

As we calculated lifelong daily dose of phthalates, we determined that its value was maximum for people who consumed milk in PET bottles (Table 3).

Table 1

Maximum concentrations of basic phthalates in milk samples in different polymer package, mg/l

Package	Phthalates concentration in milk, mg/l				
	DMP	DEP	DBP	BBP	DEHP
LDPE	0,161	0,25	1,686	0,072	3,709
PET bottle	ltdl (<0,1)	нпо (<0,2)	29,808	нпо (<0,2)	12,537

Note: * – ldl means lower than detection limit

Table 2

Maximum phthalates doses which enter a body with milk in polymer package for different groups (mg/kg of body weight per day)

Group	Maximum daily milk consumption, l	Substance	Package type	Dose
Children aged 4-6	0,2	DMP	Polyethylene film	$2{,}44 \cdot 10^{-3}$
		DBP		$2{,}55 \cdot 10^{-2}$
		BBP		$1{,}06 \cdot 10^{-3}$
		DEHP		$5{,}61 \cdot 10^{-2}$
		DEP		$3{,}78 \cdot 10^{-3}$
Children aged 7-17	0,3	DMP	Polyethylene film	$1{,}43 \cdot 10^{-3}$
		DBP		$1{,}49 \cdot 10^{-2}$
		BBP		$6{,}21 \cdot 10^{-4}$
		DEHP		$3{,}29 \cdot 10^{-2}$
		DEP		$2{,}22 \cdot 10^{-3}$
		DMP	PET bottle	0
		DBP		$1{,}50 \cdot 10^{-1}$
		BBP		0
		DEHP		$6{,}32 \cdot 10^{-2}$
		DEP		0
Adults	0,6	DMP	Polyethylene film	$1{,}82 \cdot 10^{-3}$
		DBP		$2{,}83 \cdot 10^{-3}$
		BBP		$1{,}19 \cdot 10^{-2}$
		DEHP		$8{,}15 \cdot 10^{-4}$
		DEP		$4{,}20 \cdot 10^{-2}$

Table 3

Results of lifelong daily phthalates dose calculation for different package, mg/kg of body weight a day

Substance	Package	
	Film	PET
DMP	$1{,}79 \cdot 10^{-3}$	$1{,}22 \cdot 10^{-3}$
DBP	$1{,}22 \cdot 10^{-2}$	$6{,}63 \cdot 10^{-2}$
BBP	$5{,}24 \cdot 10^{-3}$	$4{,}99 \cdot 10^{-3}$
DEHP	$2{,}47 \cdot 10^{-2}$	$3{,}68 \cdot 10^{-2}$
DEP	$1{,}84 \cdot 10^{-2}$	$1{,}76 \cdot 10^{-2}$

Table 4

Lifelong risk which occurs milk in polymer package is consumed: assessment results

Substance	Hazardous index (HI)			
	The liver		The endocrine system	
	Film	PET	Film	PET
BBP	0,03	0,02	Is not a critical system	
DBP	Is not a critical organ		0,12	0,66
DEHP	1,24*	1,84*	1,24*	1,84*

Note: * – means risk is unacceptable

In this case DBP makes the greatest contribution into this dose. As for people who consume milk in polyethylene film their dose is formed mostly due to DEHP.

At the stage when risk was characterized we detected that consumption of milk in polymer package caused unacceptable lifelong risk of hazardous effects evolvement (HI up to 1.84) in the liver and the endocrine system (Table 4). DEHP makes the greatest contribution into this risk regardless of milk package.

So, we detected that when milk in polymer and polymer-containing package is consumed, it can cause unacceptable lifelong risk which occurs due to phthalates content in milk.

Conclusions. Overall, the obtained results allowed us to reveal that:

– dimethyl phthalate (DMP), dibutyl phthalate (DBP), di-ethylhexyl phthalate (DEHP), and butylbenzyl phthalate (BBP)

occur in milk in polymer package. Phthalates were detected in 40% samples in PET bottles and in 70% samples in polyethylene film;

– 57% adults, 75% children aged 4-6, and 80% children aged 7-17 of all the respondents consume milk in polyethylene film and (or) PET bottles;

– actual daily milk consumption amounts to 0.1 to 0.6 l for adults; 0.1–0.2 l, for children aged 4-6; 0.1–0.3 l, for children aged 7–17;

– phthalates doses entering a body with milk amount to $5.61*10^{-2}$ mg/kg of body weight for children aged 4-6; $6.32*10^{-2}$ mg/kg of body weight for children aged 7-17; $4.20*10^{-2}$ mg/kg of body weight for adults;

– lifelong risk calculation allowed to reveal that risk occurred due to DBP when milk in polyethylene film and PET bottles was consumed. Risk-characterizing hazardous indexes reach 1.84 for the liver and the endocrine system regardless of package.

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