„BABEŞ-BOLYAI" UNIVERSITY, CLUJ-NAPOCA

COLLEGE OF ENVIRONMENTAL SCIENCE AND ENGINEERING

DOCTORAL SCHOOL: ENVIRONMENTAL SCIENCE

MODEL OF EVALUATION OF HUMAN EXPOSURE TO PHTHALATES

- PhD THESIS SUMMARY-

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Keywords: phthalates, method validation, drinking water, bottled water, phthalates metabolites, daily intake, behavior analysis

Note: When drafting the summary they were kept the same notations for chapters, tables, formulas and figures used in the text of the thesis.

1. Framework and hypothesis

Humans are exposed to a variety of chemicals due to pollution sources that are becoming more and more diverse. The dynamic character and variability of the exposure to chemical compounds brought to discussion the issue of cumulative risk from exposure to chemical mixtures. As such, the US Environmental Protection Agency (EPA-US) published the "Guidelines for the Health Risk Assessment of Chemical Mixtures" in 1986. Over time, the US EPA has published a number of framework papers and documents concerning risk assessment associated with cumulative exposure (ILSI 1999; EPA, 1997, 2000, 2002, 2003, 2006, 2007). Some of these documents were focused on classes of chemicals or substances with similar chemical and physical properties, on the assumption that they have a common mechanism of action, e.g. dioxins (EPA, 1993; Van den Berg et al., 2006), polychlorinated biphenyls (Van den Berg et al., 2006), polycyclic aromatic hydrocarbons (EPA, 1993), organophosphorus pesticides (EPA, 2002) etc.

Phthalates are another class of substances for which the US EPA asked the National Research Council (NRC, 2008) the opportunity to conduct a cumulative assessment of these chemical classes and to provide guidance for the exposure risk assessment, but also for assessing the cumulative exposure to phthalates and other chemical compounds. In this request, the US EPA focused on the following eight phthalates: dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), butyl benzyl phthalate (BBzP), di-n-pentyl phthalate (DnPP), di (2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DnOP), diisononyl phthalate (DiNP) and diisodecyl phthalate (DIDP) (EPA, 2012).

Phthalates are a class of synthetic compounds produced for the first time in 1920, after that, large-scale commercial production was introduced during the 1950s; in the mean time, polyvinyl chloride (PVC) was synthesized (Genuis et al., 2012).

The widespread use of phthalates in industry leads to an occupational and non-occupational exposure to this class of substances. The nonoccupational exposure is frequent and intense due to the contact with various products containing phthalates, including medical devices. Contaminated and water indoor air are other important pathways of entering the human body. On the other hand, the occupational

exposure is also frequent but less intense, involving smaller numbers of individuals at risk.

Literature from the past 15 years shows evidence regarding the almost ubiquitous presence of phthalates in the environment, about their properties and the changes that undergo in the environment and live organisms and last but not least regarding the multiple effects on human organs and body systems. The severity of human health effects observed in the literature includes human carcinogenesis supported by experimental studies on animals, possible for some of the components of this class.

Children are a group with particular susceptibility to chemical exposure to substances known or suspected to be endocrine disruptors, the group of substances that phthalates belong to. What is known at this time from studies on the adverse effects of endocrine disruptors (fetal growth, early development of the reproductive system, pubertal development, neurodevelopment and obesity) should be used for health recommendations, but in the same time as basis for future research (Meeker, 2012).

Based on current scientific information regarding the toxicology of phthalates I set the following assumptions:

- because of their multiple contamination sources, phthalates are present in varying concentrations in water and food, their identification and measurement requiring specific analysis methods;
- there is a human exposure to phthalates from drinking water and food, which is more frequent and intense due to changing consumption habits, and the materials that come in contact with them;
- due to simultaneous exposure to different phthalate sources, the presence of their metabolites in urine (biomarkers of effect) is related to the frequency, duration and intensity of the exposure.

The aim of this study is to clarify these assumptions by achieving the following objectives:

- adjusting the analysis methods of phthalates from liquids and biological matrices;
- researching the transfer of phthalates from packaging to content (bottled water, food);

- determining phthalates content from public drinking water supply systems;
- measuring human phthalates exposure biomarkers (their metabolites from urine);
- calculating the integrated human exposure to three phthalic acid diesters;
- a better information of the scientific environment on the effects caused by water and food contamination with xenobiotic substances (possibly carcinogenic) while improving their specific analytical methods.

2. Documentary study and review of the literature on the effects of phthalates on health

This chapter contains the documentary study and review of the literature on the prevalence of phthalates in the environment and their effects on health divided into seven chapters: structure and physicochemical properties of phthalates, their fate in the environment, areas of use, methods of analysis, human exposure to phthalates, biomarkers of human exposure, regulations and reference doses.

3. Experimental design

The research study has two distinctive and successive parts aimed to verify the hypothesis and achieving the objectives.

The first part of the research consists in the study of analytical methods used for identifying and measuring the concentration of phthalates in various matrices by developing gas chromatographic methods. It also includes the development of experimental models and analytical techniques for highlighting phthalates transfer from products containing plasticizers (plastic packaging for beverages, food bowls, water pipes) in water and food and the factors favoring these transfers.

The second part of the research consists in the assessment of human exposure to phthalates by analyzing their metabolites (biomarkers of exposure). A pilot study was conducted to investigate the exposure to phthalates through water and food by identifying the urine metabolites from 43 subjects and a survey about their exposure to these compounds.

Based on this pilot study, a biomonitoring model was developed by investigating a new group of 25 subjects and identifying and quantifying metabolites in urine. They filled in a specific and complex questionnaire regarding individual source and exposure pathways of phthalates.

Based on the metabolites concentration in urine, the daily phthalates intake and health effects forecast were calculated by taking into account the frequency, duration (based on the risk score obtained from the questionnaire) and intensity of exposure by ingestion as a result of individual consumption of drinking water.

Experimental models have been generated in terms of a consumer's exposure that purchases a known, reputable, authorized/approved for marketing both in terms of content and product packaging, kept and displayed for sale in proper conditions. Therefore, the experimental conditions have reproduced the usage and storage conditions of an average person for both water and food.

4. Personal results

Subsection 4.1. describes the analytical methods used and the validation of analytical methods.

Sample analysis for the determination of phthalates in various matrices requires laborious preparation of glassware used in the analysis to avoid contamination of samples.

In addition to the determination of analytes of interest in liquid samples and biological matrices water samples were analyzed for pH and urine samples for creatinine.

Analysis of phthalates from water

The standardized method described in SR EN ISO 18856/2006 is selective, sensitive and suitable for our purpose to analyze the liquid matrix for the determination of phthalates. The method involves the extraction of compounds from water by solid phase extraction (SPE), their separation by gas chromatography with the use of capillary columns followed by identification and quantification of phthalates by mass spectrometry.

The advantages of this method are the short processing time of the sample and the small volume of the solvent used, but requires special equipment for performing the extraction.

Once we found the retention time of each phthalate and oven temperature program suitable for good separation of the peaks, tests were made to find the optimal parameters for an increased signal intensity and a better signal/noise ratio.

For this, solutions of known concentration were analyzed and various parameters have been changed like injection pressure (Figure 7), temperature of the injection (Figure 7) and carrier gas flowrate.

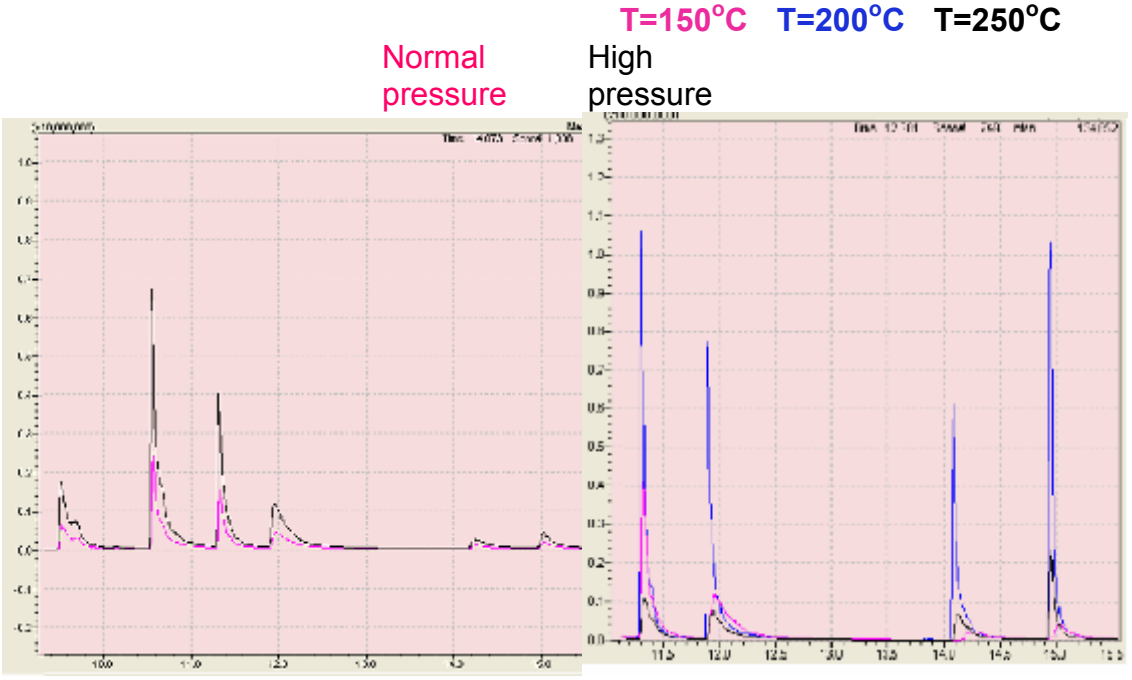


Figure 7. Chromatograms of phthalates from water analysed in various conditions

A significant parameter influencing the signal strength is injection pressure. There was an increase in signal intensity when using a high pressure injection during the analysis. Also, injection port temperature significantly influences the signal strength. If it is too small, high molecular weight compounds will not vaporize sufficiently.

Quality control was conducted by analyzing blank samples of distilled water and by analyzing a standard solution with a concentration of 0.08 µg/l.

The control chart shown in Figure 10 shows that the process is in statistical control, all control values are within the warning limits.

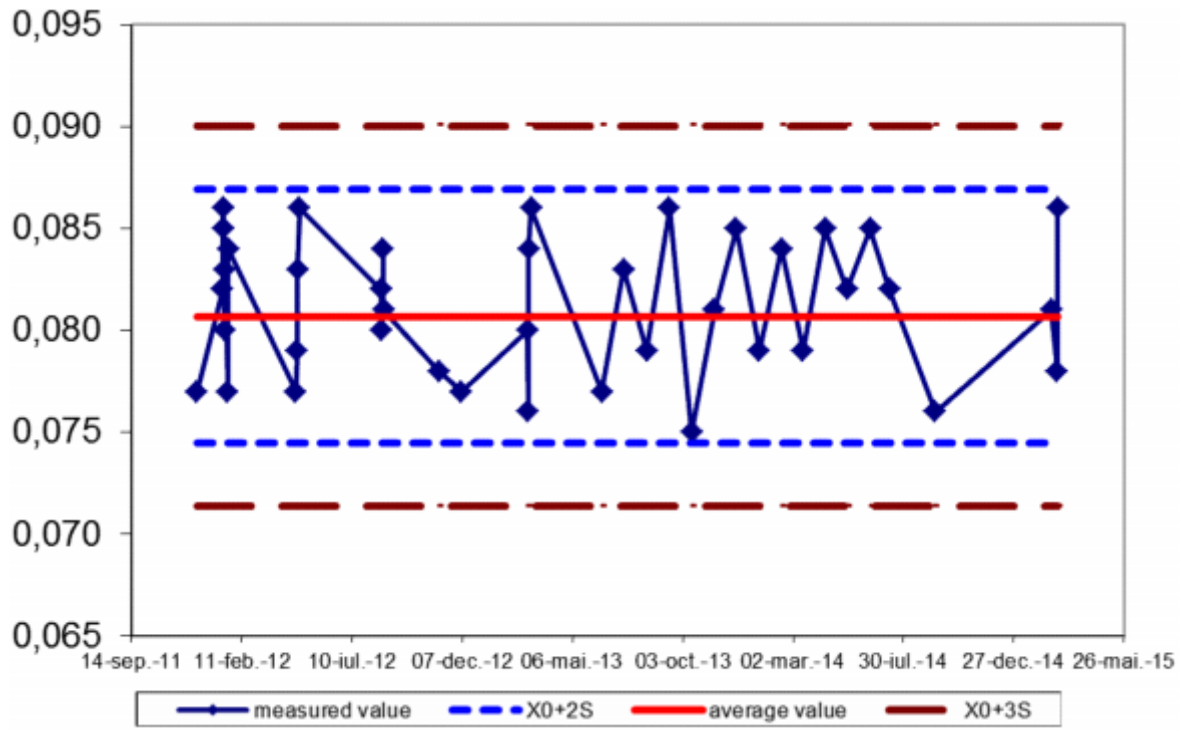


Figure 10. Type X control chart for DBP

The experimental results obtained for the determination of the phthalates from water, have proved that the method is suitable for the intended purpose because it is linear, accurate and precise for all the compounds.

• method proved to be linear over the concentration range 0.02 to 0.14 µg/l, by conducting verification tests for homogeneity of variances and linearity;

• method is precise, which results in proving the repeatability;

• method is accurate, as demonstrated by calculating the bias of the dynamic range.

Analysis of phthalates metabolites from urine

The method for measuring the concentration of phthalates metabolites in urine involves enzymatic hydrolysis, derivatization and quantification by gas chromatography.

To better understand the concept of human biomonitoring and especially the enzymatic hydrolysis process and the complexity of the analyses from biological

matrices, a two weeks training was performed in June 2011 in the biomonitoring laboratory of the Wadsworth Center, USA (I New York State Public Health Department). In this preparation program, phthalate metabolites analysis were performed following the steps of enzymatic hydrolysis, automatic solid phase extraction and their quantification by liquid chromatography, on unknown samples sent to different laboratories in an European biomonitoring program (Dumitraşcu & Gurzău, 2011).

Approach of high performance liquid chromatography in tandem with mass spectrometry is preferable because of the lower sensitivity of gas chromatography method for phthalates metabolites. Due to the analytical limitations of the laboratory, this approach was not possible and the derivatization of compounds after enzymatic hydrolysis, followed by gas chromatography analysis were chosen. In order to achieve accurate quantification the internal standard dilution method was used.

4.2. Experimental models of phthalates transfer to liquide matrix

4.2.1. Phthalates transfer from packaging to bottled water

Due to lack of covalent bonds between polymer and phthalates, phthalates can easily pass the plastic matrix (Serrano et al., 2014) in food. Consequently, there is migration of phthalates from plastic packaging to bottled drinking water, which is a high risk factor for human health (Bach et al., 2012; Bach et al., 2013).

Assuming that bottled drinks increased intake of phthalates in the human body we conducted an experimental study to measure their level in bottled water packed in polyethylene terephthalate (PET) under different storage conditions.

Materials and methods

The study was conducted from December 2011 to January 2012 and was performed on bottled water from five producers. Samples were purchased off the shelf at a local store and random. They were transported to the laboratory and analyzed immediately.

Of the five water samples two were of still water and three sparkling water. Each sample was divided into three samples (Figure 11). A sample was analyzed

immediately after purchase and opening the package. The second sample was stored in a refrigerator (at 4°C and darkness), coded with the letter R in figures and graphs, and the third sample at room temperature (an average of 20°C), near a source of heat and light, coded in figures and graphs with the letter C near the water sample number. These two samples were kept under these conditions for 25 days (Dumitraşcu, 2012).

Analyzed immediately

Analyzed after 25 days

Figure 11. Sample division

Results and discussion

Phthalates concentrations of the samples analyzed immediately after buying the product and opening the packaging are shown in Figure 12.

Of the five phthalates analyzed four were identified (DBP, DiBP, DEP and DEHP) above detection limit, the concentration of BBzP being less than the method detection limit (Dumitraşcu, 2012). Therefore, we will further refer to the concentrations of the four phthalates quantified. Among the analytes of interest analyzed, the samecompounds in terms of identity and number were identified in the samples regardless of storage conditions and time of analysis.

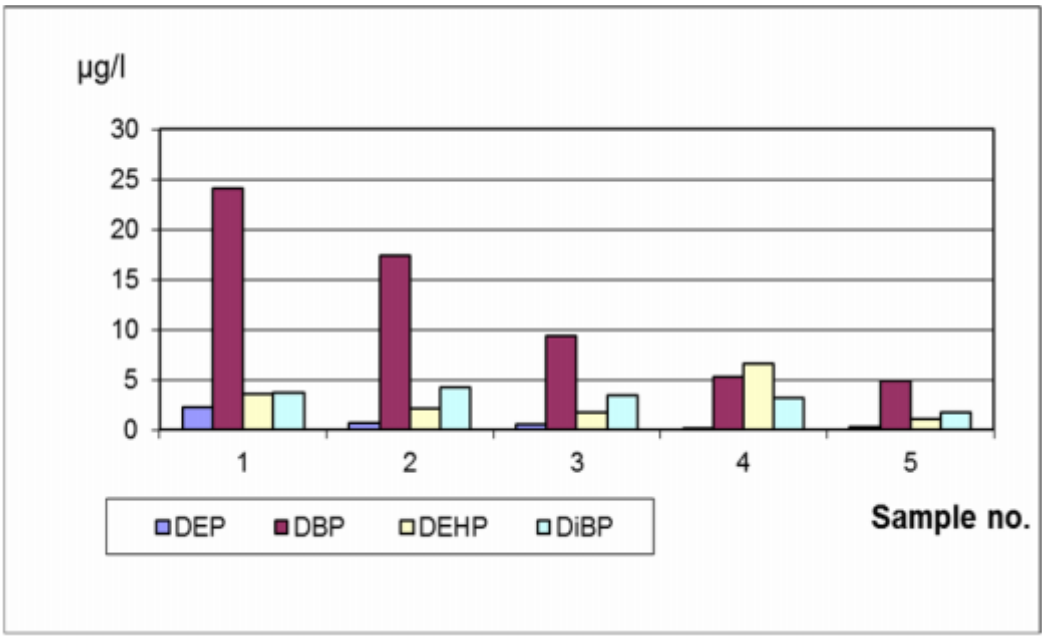


Figure 12. The concentration of phthalates at purchase

Total concentrations of phthalates in the samples analyzed and the growth rate of their storage conditions are shown in Figure 13 (Dumitraşcu, 2012) and were between 7.86 and 33.51 µg/l immediately after purchase and unpacking; between 8.72 and 42.49 µg/l when stored in cool and lightless conditions; between 11.22 and 62.10 µg/l when stored at room temperature and lighting conditions, observing an increase in concentration during storage in all cases.

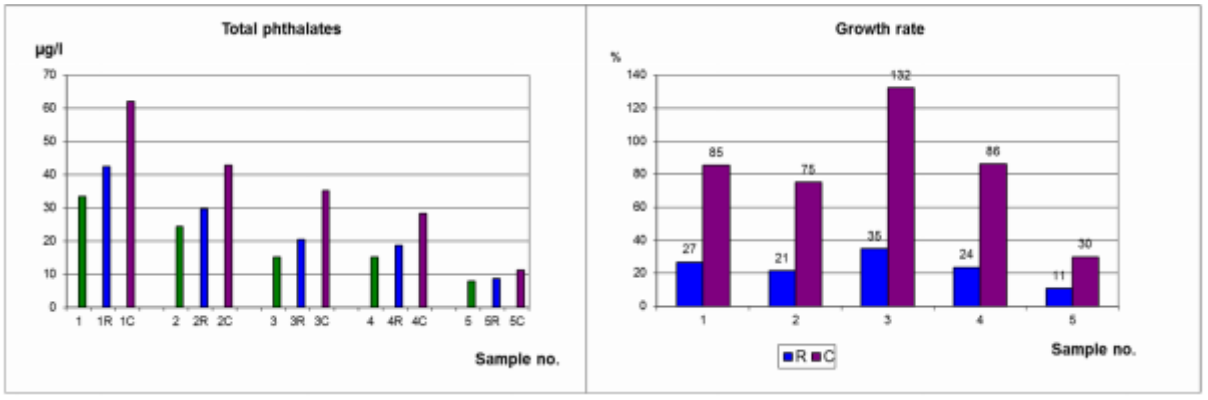


Figure 13. The total concentration of phthalate and the growth rate in different storage conditions

The highest concentrations were measured in the still water sample 1 which is a still water, characterized by an alkaline pH. In this case the highest concentrations of phthalates were recorded no matter if stored in cool and dark conditions or kept

warm. The contrary to this situation is observed for sample 5 which is carbonated water characterized by an acidic pH, but increased total concentration of phthalates was less marked relationated to storage conditions and time of analysis.

In still water samples there was a growth rate higher than in carbonated water samples, which leads us to believe that pH plays an important role in the transfer of phthalates from PET in water.

In terms of health risk (possibly carcinogenic) we further detail the modification of DEHP concentrations in water samples and storage conditions. Figure 14 evidently shows the increase in concentration for the samples stored at room temperature, in particular in the case of samples 1 and 4.

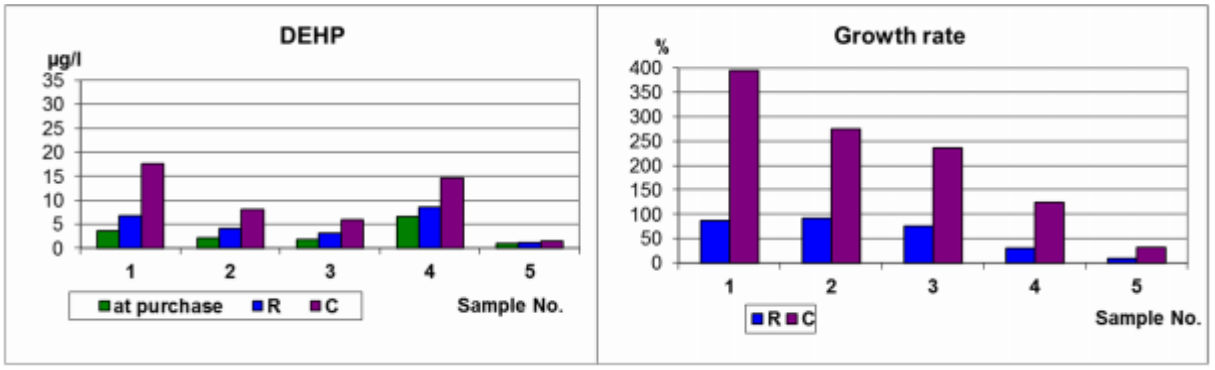


Figure 14. DEHP concentration and growth rate in different storage conditions

The highest concentrations at purchase time were measured for DBP in all samples. What is remarkable is that for each of the compounds identified concentrations were higher after keeping the sample for 25 days, regardless of storage conditions.

Growth rates take into account the measured concentration in water when opening the package without any available information on storage conditions, duration and exposure conditions on the shelf. On the other hand we do not know the concentration of these phthalates in the packaging. All interpretations are based on the concentration when opening the package.

Similar studies measuring the concentration of phthalates in bottled water show different values than ours from once the pakaging was opened (Guart et al., 2014). After having analyzed seven bottled water brands (6 PET and 1 glass containers), portuguese researchers have concluded that, although all waters contain three phthalates (DBP in concentrations of 0.06 to 6.5 µg/l, DEHP within 0.02

to 0.16 µg/l and DiBP in concentrations of 0.1 to 1.89 µg/l), water bottled in PET containers contains five times more DEHP than water bottled in glass containers, which has a concentration of DBP greater than water in PET containers (Santana et al., 2014). Montuori and his colleagues concluded that still water contains a greater amount of phthalates than sparkling, and also that bottled water in PET containers come to have a concentration 20 times higher than glass bottled water (Montuori et al., 2008).

In terms of public health, DEHP presence is significant given that it is a possible carcinogenic, especially because the European Union and Romania have not regulated its concentration in drinking water. This compound is the only phthalic acid diester presentin water in the United States regulated by the US EPA (EPA, 2009) at a maximum concentration of 6.0 µg/l. Referring to this regulation, our analyzed samples fall below the above mentioned limit at purchase time, when opening the package, with the exception of sample 4, which had a value of 6.53 µg/l. Storage for 25 days at room temperature led to exceeding the limit in 4 of the 5 samples analyzed. If we consider the recommendations of the World Health Organization (WHO, 2008), DEHP concentration for all water samples analyzed at the time of purchase was below the maximum permissible value (8.0 µg/l), but this recommended concentration was exceeded during storage conditions at room temperature for 25 days after opening the package (Dumitraşcu, 2012).

4.2.2. Phthalates transfer from catering packaging to liquid foods

According to the literature foods seem to be a major contributor (95.5%) for phthalate intake by digestive path (Martine et al., 2013).

Due to the growing trend of consumption of plastic-packaged foods, we propose an experimental model for determination of phthalates transfer into liquid food.

Materials and methods

Considering the consumption of liquid food provided in catering type package and especially because most often these foods are heated in the delivered package, we assume that phthalates transfer to the contents of the package. Thus, we

simulated conditions using distilled water instead of liquid food with correction of predisposing factors (in this case the pH).

Compounds for which was determinated the concentration in the polystyrene food packaging are di (2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), di (isobutyl) phthalate (DIBP) and butyl benzyl phthalate (BBzP).

The distilled water used was analyzed as a control sample to eliminate possible contamination due to extraction and concentration procedures or materials used.

Results and discussion

To determine the experimental conditions we measured the pH (Table 14) according to the method described in section 4.1.1. for a total of 14 liquid foods supplied by the caterer.

The experimental environment was created by acidification of 300 ml of distilled water with acetic acid and transfer to polystyrene catering type containers (figure 18). We have created five samples with different pH values covering the range measured for soups delivered.

Figure 18. Catering type containers

The samples thus prepared were allowed to stand for 3 hours at room temperature and heated in a microwave oven similar to soups heating time (1.5 minutes), then analyzed in the same way as water samples.

We have identified three of the four analized phthalates (DiBP, DBP, DEHP).

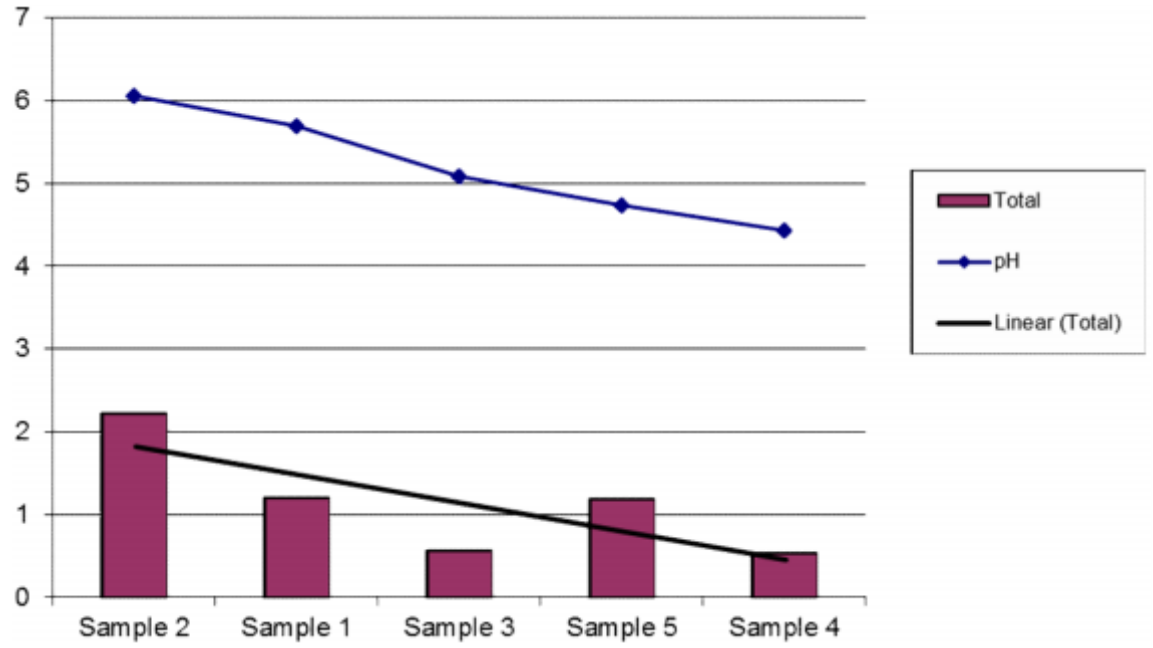


Figure 19. Correlation between pH and the total concentration of phthalates

There is a parallelism in the evolution of concentration and pH values which leads us again to conclude that alkaline pH is favorable for phtalate transfer (Figure 19). Correlation is better at higher pH values than at lower ones.

Concentrations, although small, demonstrate that phthalates are mobilizing even if the contact time is reduced (in the order of hours).

4.3. Case study on the concentration of phthalates in public drinking water systems

European Commission requirements for the development and improvement of drinking water supply infrastructure has led to the establishment of numerous projects of central supply systems in rural areas and the expansion and modernization of those in the urban areas.

International and national regulations lay down specific and severe conditions regarding the use of products that come in contact with drinking water. Assuming that phthalates, used as plasticizers in the composition of the material used to produce drinking water pipes, can migrate to content under certain conditions we intend to analyze four compounds from drinking water (dibutyl phthalate, diisobutyl

phthalate, di-2-(ethylhexyl) phthalate and benzyl butyl phthalate) knowing that: DEHP and BBzP are used in the composition of water pipes manufactured from polyvinyl chloride and two (DBP and DiBP) which we identified in previous studies (Dumitraşcu, 2012) in bottled water packaged in polyethylene terephthalate having the same basic monomer as high density polyethylene pipes (HDPE). Also our research started on assumption that the information in the literature regarding phthalate transfer from pipes based on high density polyethylene are few (Bagel-Boithias et al., 2005).

4.3.1. Rural supply system - modernization of water supply in rural areas

Study area, material and method

We investigated six water supply systems in rural areas from Cluj and Hunedoara counties with different operating periods:

- Alunișu treatment system, Sâncraiu village, Cluj County;
- Călata treatment system, Călăţele village, Cluj County;
- All four systems of the localities Feregi, Poieniţa Tomii, Cerbăl and Socet from Cerbăl village, Hunedoara County.

A common feature of these systems are the water supply sources, which are captured springs.

Another common feature is that all pipes in the system are of high density polyethylene.

Among these central supply systems for drinking water two of them use the conventional treatment technology, coagulation-decantation-chlorine disinfection, whereas the others treat the water only by chlorination in the tank.

Phthalates were determined for each of the investigated systems from the water source, distribution network and where was possible, from the water treatment station output (in Călata village).

Each drinking water supply system was analyzed according to law 458 republished in 2012 for chemical and indicator parameters.

Results and discussion

a. Phthalates

The analysis results are shown in Table 16 for Cluj county and Table 17 for Hunedoara county.

	Călata raw water	Călata treatment station output	Călata network	Alunişu raw water	Alunişu network
DiBP µg/l	< 0.015	< 0.015	**0.053**	< 0.015	< 0.015
DBP µg/l	< 0.015	< 0.015	**0.052**	< 0.015	< 0.015
BzBP µg/l	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015
DEHP µg/l	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015

Table 16. Cluj county results

	Poienita Tomii raw water	Poieniţa Tomii network	Feregi raw water	Feregi network	Cerbăl raw water	Cerbăl network	Socet raw water	Socet network
DiBP µg/l	< 0.015	**0.337**	< 0.015	**0.094**	< 0.015	**0.091**	< 0.015	< 0.015
DBP µg/l	< 0.015	**0.072**	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	**0.283**
BzBP µg/l	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015
DEHP µg/l	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015

Table 17. Hunedoara county results

In Călata village raw water was deprived of compounds that we analyzed (DBP, DiBP, BBzP and DEHP), the same situation being identified at the treatment station ouput. Lack of phthalates in completely treated water is because the station is monoblock type, distance traveled and time required for water treatment process are short.

We have identified two compounds at similar concentrations DiBP (0.053 µg/l) and DBP (0.052 µg/l) in the distribution network. We consider that the presence of these compounds is owed to the transfer from distribution network material and contact time between the water and pipeline (the distance between the Călata treatment station ouput and the sampling point is 3 km).

The investigated compounds in Alunişu (table 16) were not identified either in raw water (Zanda spring) nor at the end of the distribution network, the distance between the tank and this sampling point is about 3 km.

The central water supply systems were investigated in localities belonging to the commune of Cerbăl in Hunedoara county (table 17) for the content of phthalates in raw water and distribution network. Poieniţa Tomii locality was the only one of the four studied localities where two compounds were identified, DiBP (0.337 µg/l) and DBP (0.072 µg/l). The network water of the other three localities, with no detectable concentrations of phthalates in raw water as well, contained only one compound, namely: Feregi - DiBP (0.094 µg/l), Cerbăl - DiBP (0.091 µg/l) and in Socet - DBP (0.283 µg/l).

We noticed that according to the results obtained, the highest concentration of DiBP was found in the distribution network of Poieniţa Tomii, with an order of magnitude higher than in all other localities, both in Hunedoara and Cluj counties. The highest measured concentration of DBP , with an order of magnitude higher than in all other localities, was in the network of Socet locality.

Other studies have reported DBP concentrations similar as order of magnitude (Serodio & Nogueira, 2005), but the type of pipe material is not specified.

Since we refer to deep water sources (water not contaminated with phthalates, as it resulted from the analyzed samples), even these low concentrations clearly show the existence of the compound in the pipe material and its transfer to drinking water, however in different proportions. In order to check this aspect we calculated the concentration increase of the compounds identified in the distribution network in comparison to raw water (Figure 21).

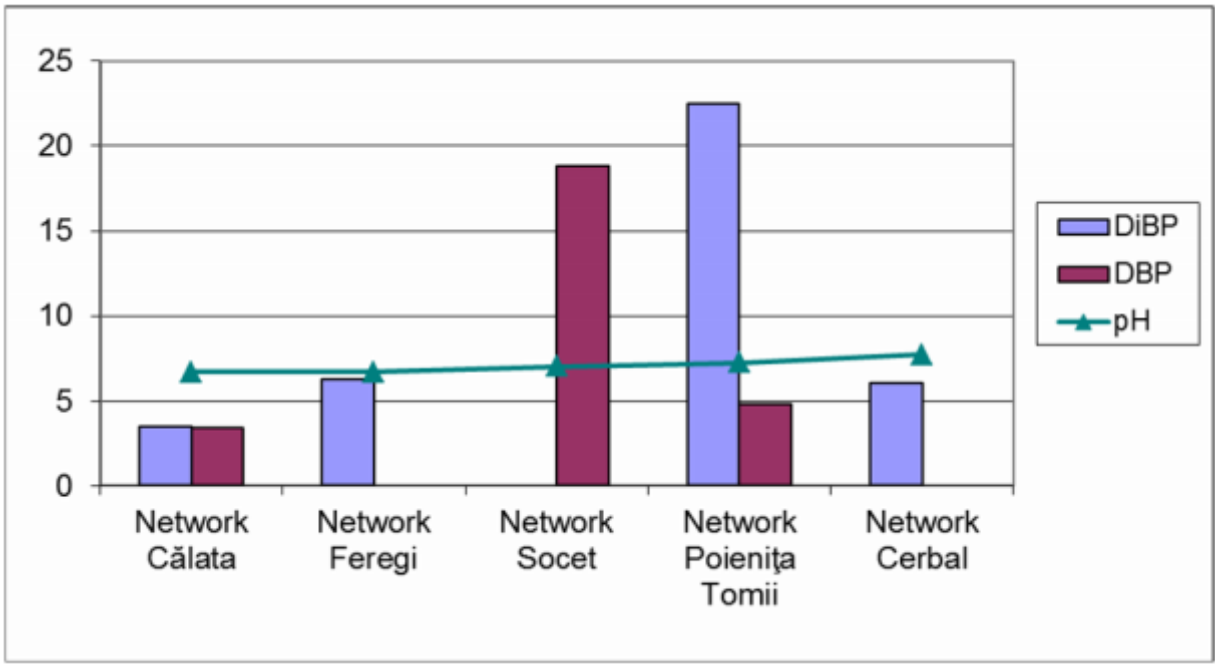


Figure 21. Growth rate of phthalates in the distribution network and pH of samples

Chart 21 shows the highest growth rate of DiBP in Poieniţa Tomii locality, followed by Cerbăl and Feregi localities that had approximately the same growth.

The fact that we have not identified compounds known in the literature as constituents of PVC-based water pipes, does not mean that they do not exist in the composition of high density polyethylene pipes. Bagel-Boithias et al. (2005) showed that DEHP is mobilized from PVC pipes, but not from HDPE pipes. Our investigations have not identified scientific studies that relate to the relationship between phthalates and water in the case of high density polyethylene pipes.

b. Water quality investigated according to law 458 R1

Control and audit monitoring according to law 458 R1 regarding drinking water quality provides information with respect to the possible health hazards of some substances/compounds with certain effects. In the case of synthetic compounds such as phthalates, excepting DEHP, whose specific effects, other than the group effect as endocrine disruptor, are not currently known , there are no rules or recommendations regarding the maximum allowable concentration in drinking water. Their determination in water may constitute in scientific arguments regarding at least a qualitative regulation for materials in contact with water, but without being considered a routine monitoring regarding their content in water.

4.3.2. Urban supply system - central water supply system in Cluj-Napoca

We proposed to extend the research by determining the phthalate content of water in different areas of Cluj-Napoca municipality considering the diversity of pipe types in the network (materials used), the modernization works of the network and the replacement of the existing pipes with HDPE pipes and not least considering the raw water quality and its treatment system.

Area of study, materials and method

The regional water supply source for Cluj-Napoca municipality and other nearby localities is a surface source (Tarniţa Lake). Tarniţa reservoir is part of a system of lakes used mainly for hydroelectric purpose on the course of Someşul Cald River. The area of these lakes is on the other hand one with permanent rural localities and a tourist area developed mainly after 1995. Like any surface water source these lakes are vulnerable to anthropogenic contamination, waste plastics (PET) being present mainly in the dams area (Figure 22). Water intake is located at 20 m below the water surface.

Figure 22. Tarniţa Lake - Gilău water treatment plant - Cluj Napoca

The water supply system of Cluj Napoca municipality is composed of:

- Tarniţa dam with the adduction inlet;
- Adduction pipe made of fiberglass over a distance of 7 km, partially crossing underwater Someşul Cald Lake;
- Gilau water treatment plant;
- Water tanks in the city;
- Mixed distribution network to consumers.

Inside the water treatment plant all water transport pipes between technological steps are made of concrete or steel.

The regional water operator proceeded and further proceeds with the replacement of old steel pipes with high density polyethylene pipes.

A total of 32 water samples were collected. Sampling was performed in two phases, 16 samples in September 2014 and the same number in February 2015.

We proceeded to the collection of water samples in the following points: Tarniţa Lake dam (surface sample) and on the technological steps on the three lines of treatment: raw water, decanted water, fully treated water, from the distribution network of ClujNapoca municipality, seven sampling points evenly distributed and representative for urban supply areas and from the distribution network of Floreşti locality. This is the first village near Cluj-Napoca towards West, where in recent years residential estates were built, whose residents work mostly in ClujNapoca and whose infrastructure for drinking water supply from the public network is new in the area where sampling was performed (about 8 years) and is made of high density polyethylene pipes (Figure 25).

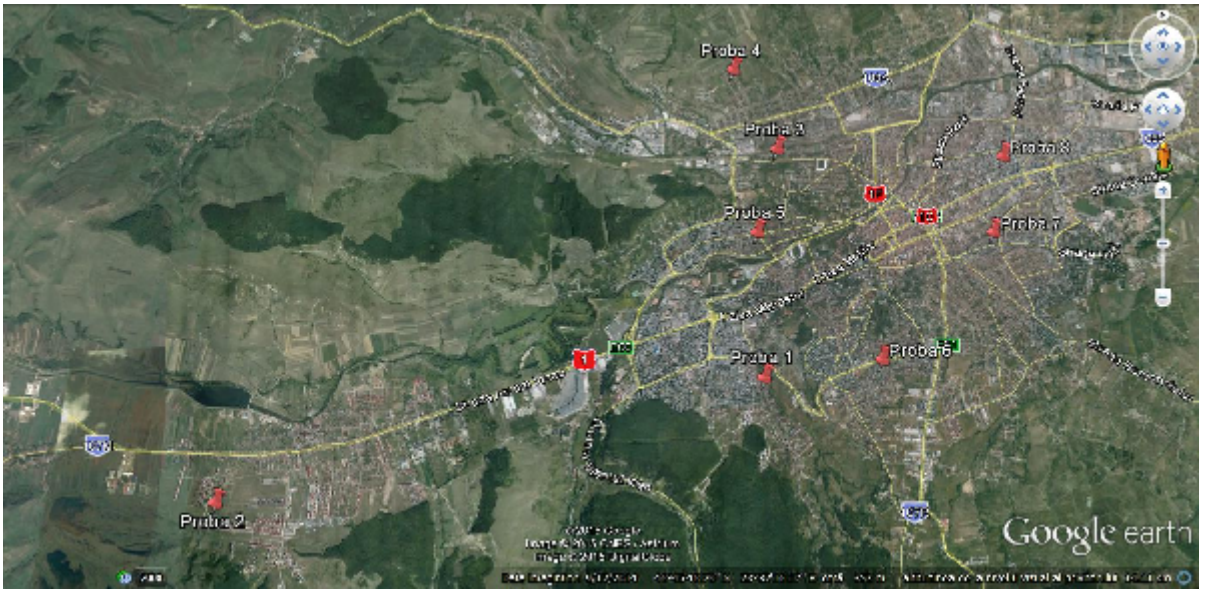


Figure 25. Sampling points in the city.

Results and discussions

A. Tarnita Lake

Two (DiBP and DBP) of the four compounds analyzed were identified in the surface water sample collected from the Tarnita Lake dam area. The presence of phthalates in the surface water area can be explained by the existence of numerous PET residues at the sampling site. Sampling was performed on purpose in this place to see to what extent PET residues influence the concentration of phthalates in water.

Dibutyl phthalate presented the lowest concentrations of the measured values of the compounds analyzed (0.097 µg/l towards 0.103 µg/l for DiBP). Dibutyl phthalate was not identified again in the sampling campaign in February, 2015 (Table 32).

As shown in Table 32, DiBP concentration in Tarnita Lake was three times higher in February 2015 (0.334 µg/l) than in September 2014 (0.103 µg/l), which is explained by the lack of heat and ultraviolet radiation, contributing factors to the biodegradation of phthalates.

	Sep.2014	Feb. 2015
DiBP (µg/l)	**0,103**	**0,334**
DBP (µg/l)	**0,097**	< 0,015
BzBP (µg/l)	< 0,015	< 0,015
DEHP (µg/l)	< 0,015	< 0,015

Table 32. Phtalate concentrations in Tarniţa Lake.

Contrary to expectations, DEHP was not identified (which is standardised in Romanian law to 1.3 µg/l in surface waters) given that there were numerous PET residues in the water and another study showed its transfer from the packaging in the bottled water (Dumitraşcu, 2012). As in the case of other compounds, DEHP transfer and biodegradation are interrelated with the pH of water, presence of solar radiation and temperature (Dumitraşcu, 2012).

B. Gilau water treatment plant

Water samples collected at Gilau water treatment plant on technological steps were also characterized physically and chemically (data obtained afterwards from the laboratory of Gilau water treatment plant belonging to the regional operator, Someş Water Company S.A., for the sampling day and the next day) (Table 33).

The consistency of drinking water quality with the national and European regulations for the presented parameters is demonstrated by determining the usual parameters. The analyzed parameters allow us to characterize raw water as a water with low turbidity, slightly alkaline pH, with a low content of salts and a very low load of nitrogen-containing substances (ammonia, nitrites and nitrates).

With regard to completely-treated water, analysis of the same parameters in the three water tanks shows that water preserves the characteristics mentioned above.

For raw water treated at Gilau water treatment plant (adduction inlet is at 20 m below water surface) only one compound out of the analyzed ones was identified (Figure 26), DiBP in concentration of 0.047 µg/l in February 2015 and DBP in concentration of 0,027 µg/l in September 2014, both being lower than at the lake surface.

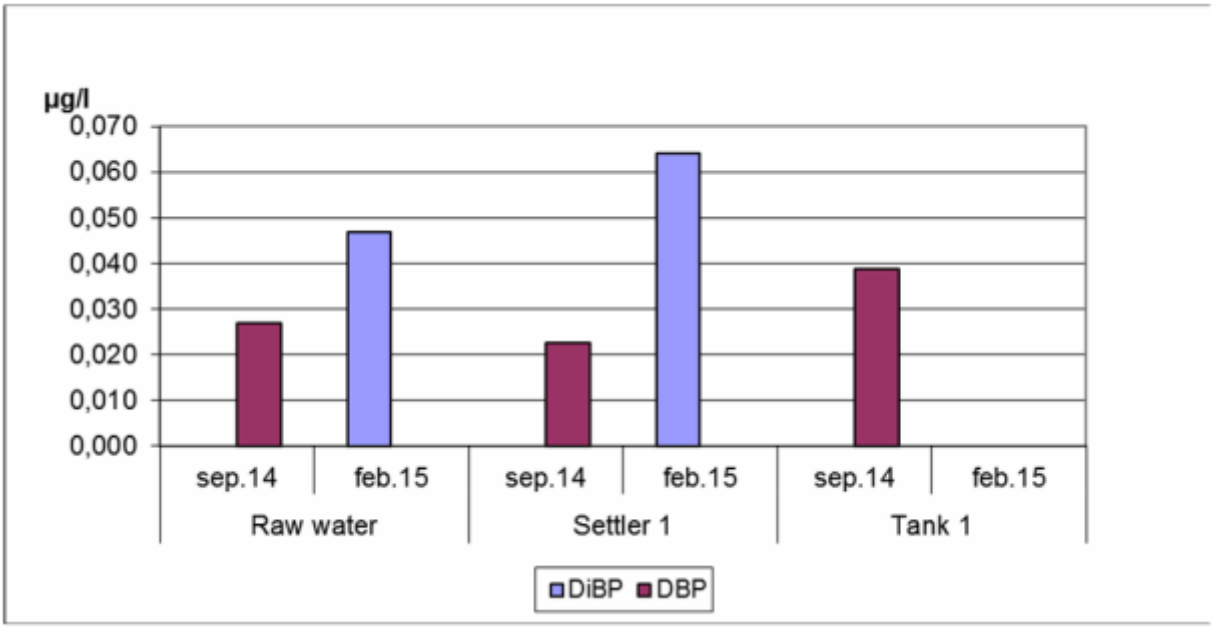


Figure 26. Phthalate concentrations during the treatment process

Since the adduction from the water inlet to the water treatment plant where we collected the raw water sample for analysis is, as noted, made of fiberglass and

therefore we exclude contamination with phthalates en route, occurence of these compounds in raw water is due, most likely, to the PET residues in the water both in Tarniţa Lake and in the upstream lakes, ascending and descending currents carrying to the depth a part of the phthalates present at the surface that no longer can degrade.

Of the four analyzed compounds we could only identify DBP during the sampling campaign in September 2014 and only in technological line 1, water decanter 1 and water tank 1, respectively. We identified only DiBP in February 2015 in decanter 1, with a higher concentration than in raw water (Figure 26).

The only published study we have found concerning the influence of water treatment process on the concentration of substances with endocrine disruptive potential in drinking water is that of Li et al., 2010, which showed that these substances are not removed effectively from the water during the treatment process. The study also concluded that DBP contributed with 50-100% to the toxicity equivalency factor in the associated experimental study.

C. Distribution network

Of the three completely-treated water tanks corresponding to technological lines, water enters the main transport pipeline towards downstream localities, this pipeline being made of prestressed concrete premo type. From the city water tanks to consumers, the distribution network is mixed in terms of materials used, being made mainly of steel and high density polyethylene.

The results in Table 35 show that out of the four compounds, DiBP was found during sampling both in September 2014 and in February 2015, while DBP was found during sampling only in one point in September 2014.

Sampling point	DiBP		DBP		BzBP		DEHP	
	(µg/l)		(µg/l)		(µg/l)		(µg/l)	
	11.09. 2014	24.02. 2015	11.09. 2014	24.02. 2015	11.09. 2014	24.02. 2015	11.09. 2014	24.02. 2015
Manastur	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015
Floresti	**0.105**	**0.201**	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015
Gruia	< 0.015	**0.061**	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015
Dambu Rotund	**0.308**	**0.128**	**0.044**	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015
Grigorescu	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015
Zorilor	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015
Gheorghieni	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015
Marasti	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015	< 0.015

Table 35. Phthalate concentrations in the distribution network.

We have calculated a score, we called risk score, by adding the concentration of the two compounds and observed that for September 2014 sampling, the water sample from Dâmbu Rotund had the highest risk score, followed by the water sample from Floreşti village. The situation is reversed for the February 2015 sampling, where the highest risk score was in Floreşti followed by Dâmbu Rotund.

The only compound regulated in Romania for surface waters and recommended by the World Health Organization to be monitored in drinking water is Di (2-ethylhexyl) phthalate (WHO, 2008), which was not detected in any of the drinking water samples analyzed.

4.4. Biomarkers of human exposure to phthalates

In the report of the U.S. Consumer Product Safety Commission developed in 2014, based on existing scientific data, human biomonitoring is defined as an integrated measure of exposure from multiple sources and on different routes (CHAP, 2014). The same report states that human biomonitoring permits an integrated assessment of exposure even when the exposure is unknown quantitatively and qualitatively and also if the contribution of different routes of exposure is not known.

Urine is the ideal matrix to determine phthalate exposure, urinary metabolites of phthalates being measured in an increasing number of human biomonitoring studies (Koch et al., 2004b; Silva et al., 2006; Koch & Calafat, 2009; Guo et al.,

2011; CHAP, 2014). Given the fact that urine sampling is also a non-invasive method, we chose to determine metabolites from this matrix in our models.

We proposed the analysis of urinary biomarkers of phthalate exposure in volunteer human subjects. For this purpose we have developed the method of determination presented in the following.

4.4.1. Research on the method determination of phthalate metabolites in urine - pilot study

Urine samples used for the development of the method and its validation were collected from a total of 43 subjects who participated in another study performed by our team. The subjects gave their written consent for the collection of urine samples in order to determine the concentration of phthalate metabolites.

The determined phthalate metabolites were: monobutyl phthalate (MBP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (5-OH-MEHP) and monobenzyl phthalate (MBzP), the reason for choosing them being the fact that they are phthalate metabolites banned in the European Union for packaging that comes in contact with food according to European Directive 2007/19/EC.

In order to determine the optimum parameters of urinary metabolite analysis mentioned in Chapter 4.1.6, determinations in urine samples were made in different conditions. Three samples, prepared according to 4.1.6, , and the hydrolysis time was 1.5 hour, 2.5 hours and 3 hours. Also, both the amount of derivatizing agent and the derivatization time changed. Three replicates were analyzed for each situation.

It was observed that less thermostatic time is not enough for the enzymatic hydrolysis, while more time does not influence significantly this process. The derivatization time works similarly, it was observed that 1 hour is enough to complete derivatization with an amount of 100 µl derivatizing agent.

Statistical data were obtained by taking into account the formulation in µg/g creatinine analyzed according to the method described in Chapter 4.1.2.

The results obtained from the pilot study in which we have developed the method of phthalates determination allowed the identification of all five metabolites in different concentrations in the urine samples analyzed.

The concise statistical processing of concentration values among which these compounds were identified is shown in Table 36.

	MBP	MEHP	MBzP	5-oxo-MEHP	5-OH-MEHP
	µg/g cr	µg/g cr	µg/g cr	µg/g cr	µg/g cr
median	17.31	15.12	6.56	7.04	9.03
average	37.30	21.30	14.63	9.77	16.51
minimum	2.65	1.48	1.00	2.51	1.97
maximum	295.51	91.90	182.92	34.07	88.65

Table 36. Values for metabolites in urine - pilot study.

The comparison of the ranges of values (difference between minimum and maximum), showed that they were variable from 1.00 µg/g creatinine for MBzP to 295.51 µg/g creatinine for MBP, allowing us to consider that the method devised by us is applicable even if the technical performances of the available equipment do not allow further lowering of the detection limit.

4.4.2. Assessment of exposure to phthalates through biomarkers of exposure on a group of volunteer human subjects

The purpose of this study was to assess exposure to phthalates and to attempt quantification of the water consumption contribution (public network and bottled water) to the body contamination with phthalates.

Study design:

A group of 25 subjects with permanent residence in Cluj county were enrolled in the study. All examined subjects share the water supply from the same central system (source - Tarniţa Lake, Gilau water treatment plant) and work in the same institution.

Subjects gave their written consent to participate in a questionnaire-based study regarding possible sources of exposure to phthalates and to collect one urine sample in order to measure biomarkers of exposure (primary, secondary and tertiary metabolites of phthalates).

The study participants were asked not to change their habit of drinking water or other bottled beverages in plastic packaging, to consume food packaged in plastic

(bags, catering-type food containers, disposable cups) and not to change their general lifestyle within the time between the agreement to participate in the study and the urine sample collection. A part of a questionnaire validated in another study was used, modified and adapted for the purpose of our study and to the group of subjects, being administered by a specialized person trained for this purpose.

Subjects collected the urine sample from the first morning urination and transported it to the laboratory where it was analyzed the same day. Delivery of the urine sample was accompanied by filling-up another questionnaire with questions about the amount of water and packaged food or food heated in a microwave and consumed the day before the sample collection.

Results and Discussions

a) Questionnaire

The age of the 25 subjects was between 23 and 67 years old, and the group included 17 women and 8 men.

14 of the 25 subjects (56%) have been living for over 10 years in the locality of residence, and 24 of the 25 subjects (96.5%) stated that the water supply was not changed in their homes.

As regards the way of storing bottled drinks, all subjects participating in the biomonitoring study stated that at work they store them at room temperature, while at home 13.6% of those who consume keep them refrigerated.

The overall water consumption per day for our group of subjects (all types, at home and at work) is approximately 1.29 $\pm$ 0.63 l/day and a consumption of 0.16 $\pm$ 0.35 l/day for hot drinks (tea, coffee) prepared with water from the public distribution system.

In terms of consumption of prepackaged food or fast food, 80% of respondents declare a less frequent consumption than once a week at home, but in slightly higher proportion (83.3%) have such a consumption at work.

If 32% of subjects heat food in microwaves at home, 42.9% of those heat it 2-3 times a week and a similar percentage daily or less than twice a week. The situation is reversed at the workplace where two-thirds of the subjects heat food in microwaves, 81.25% of them doing this daily.

Another section of the questionnaire focused on the use of personal care products addressed equally to men and women. The responses to the questions related to the use of makeup and nail polish showed that 6 of the 17 women (35%) use them daily, and the rest with a reduced frequency. Overall, within the group of studied subjects, shampoos are used by 18 of the 25 subjects (72%) with high frequency (every 2 days). Also, creams and perfumes are used with high frequency (daily), most of the subjects (92%) using deodorant most frequently .

Other possible sources of exposure to phthalates which we asked questions about in our questionnaire are the use of PVC-type flooring and wallpaper, 92% and 76% of respondents, respectively declared that they do not have such materials inside their homes.

Questions regarding the time spent in the car and its age (plastics inside the car are known sources of phthalates) showed a relatively close division of subjects who spend less than one hour (37.5%) or 1-2 hours in the car daily (33.3%), 29.2% spend more than 2 hours in the car daily. 36.4% of the respondents declared that the car is older than 10 years.

Wearing plastic gloves was declared as "never" by 72% of the participants.

b) Biomonitoring exposure to phthalates

We monitored for the 25 subjects the same metabolites that have been determined in the pilot study regarding the elaboration of the method by liquid-liquid extraction, followed by derivatization and gas chromatography coupled with mass spectrometry.

We proposed to evaluate the relationship between the urinary concentrations of metabolites and consumption of water and food, that is why questions about behavior during the last 24 hours previous to the collection of urine sample refer only to their consumption.

Of the five metabolites monitored, 5oxo-MEHP and 5-OH-MEHP, which are secondary and tertiary metabolites of Di (2-ethylhexyl) phthalate were identified in variable concentrations in all subjects; MEHP, primary metabolite of the same compound was found in 24 of the 25 subjects (96%). MBP, primary metabolite of dibutyl phthalate was identified in 21 of the 25 subjects (80%), while MBzP, primary metabolite of butyl benzyl phthalate was identified in the lowest number of subjects, 16 of the 25 (64%).

The summary statistics of the measured concentrations of these metabolites are presented in Table 38.

	MBP	MEHP	MBzP	5-OH-MEHP	5-oxo-MEHP
	µg/g cr	µg/g cr	µg/g cr	µg/g cr	µg/g cr
median	10.09	7.23	2.97	17.49	14.07
average	16.43	8.44	2.99	20.15	20.06
minimum	0.87	2.24	0.41	2.06	2.22
maximum	53.72	19.41	10.37	58.47	66.53

Table 38. Concentrations of metabolites in the investigated group of subjects

All five monitored metabolites were identified in 15 of the 25 subjects (60%), meaning a definite exposure to three phthalates (DBP, DEHP and BBzP).

It is observed that the highest average concentrations (Figure 29) were measured for secondary and tertiary metabolites of Di (2-ethylhexyl) phthalate, 20.15 µg/g creatinine, 20.06 µg/g creatinine, respectively. Its primary metabolite (MEHP), identified to an extent of 96% had an average concentration in the investigated group of 8.71 µg/g creatinine, which makes us estimate that the group as a whole had no significant exposure in the last 48 hours.

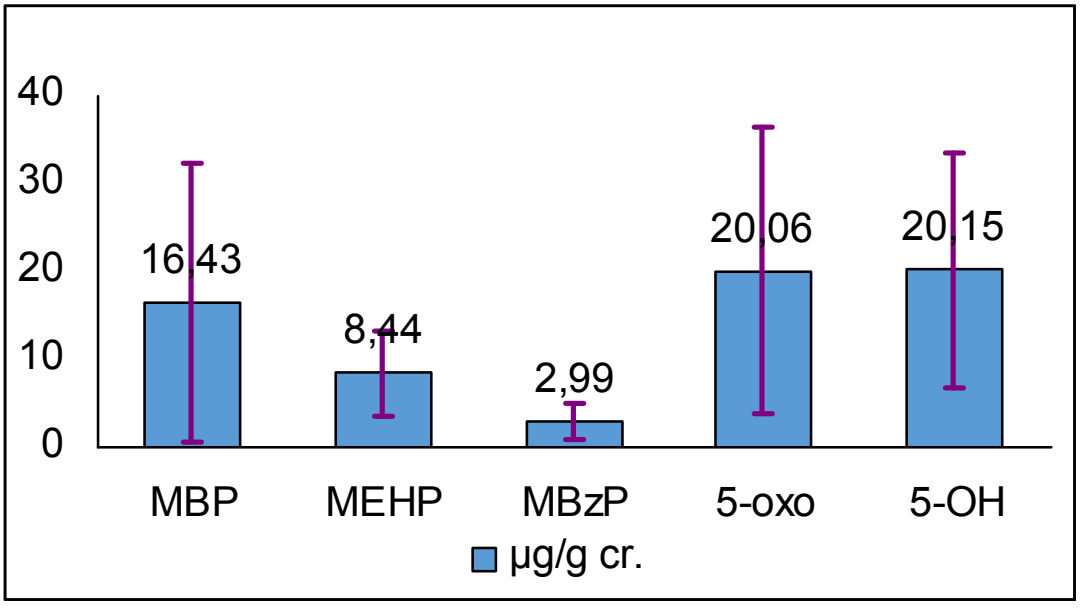
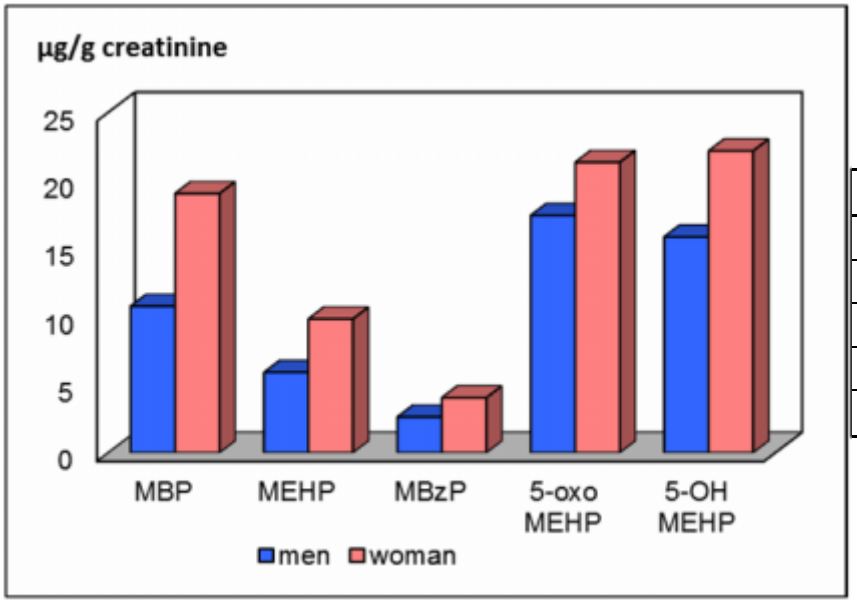


Figure 29. Average values of urinary metabolites (total)

Regarding the average concentrations of analyzed metabolites, Figure 30 shows that there are differences between men and women, being statistically significant only for the primary metabolites.



metabolite	p
MBP	**0,035**
MEHP	**0,020**
MBzP	**0,021**
5-OH-MEHP	0,099
5-oxo-MEHP	0,137

Figure 30. Average values and Student’s t-test between persons of opposite sex

Scientific literature states that phthalates are retained for a longer period of time in the adipose tissue, which is why our work comparatively presents the concentrations of these metabolites in urine according to the body mass index and depending on the results obtained we divided them into two groups: less than 25 and more than 25 (people with an index over 25 are considered overweight).

Of the eight men investigated only one exceeded the threshold of overweight, while 47% of the women exceeded this threshold. In this context we associated concentrations of phthalates in urine only for women. In Figure 31 it is observed that for DBP the differences are statistically significant (p=0.014). For women that exceed the overweight index, the average concentrations of metabolites are higher, the adipose tissue functioning as a deposit from where the release is gradual.

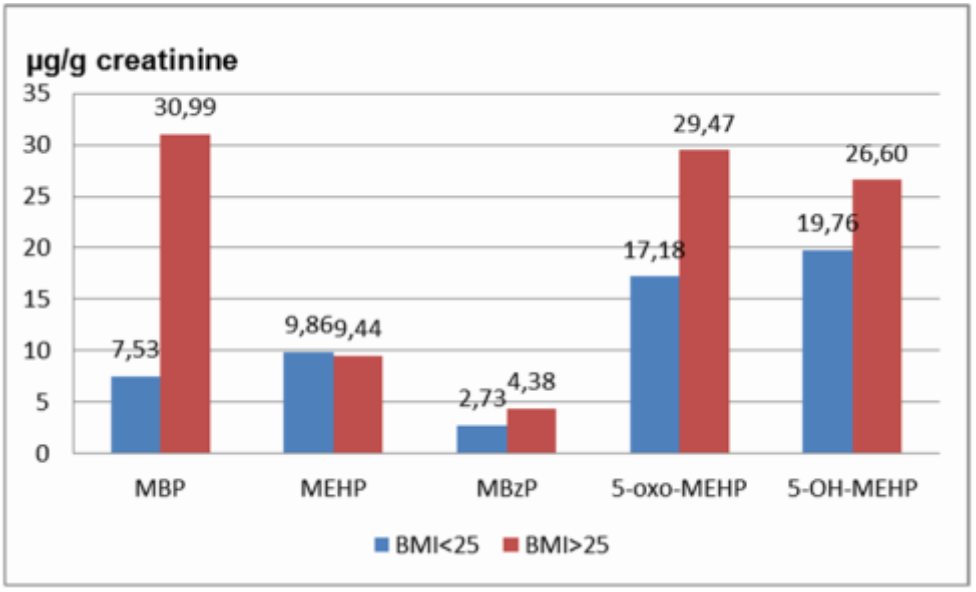


Figure 31. Concentrations of metabolites in urine depending on the body mass index for women

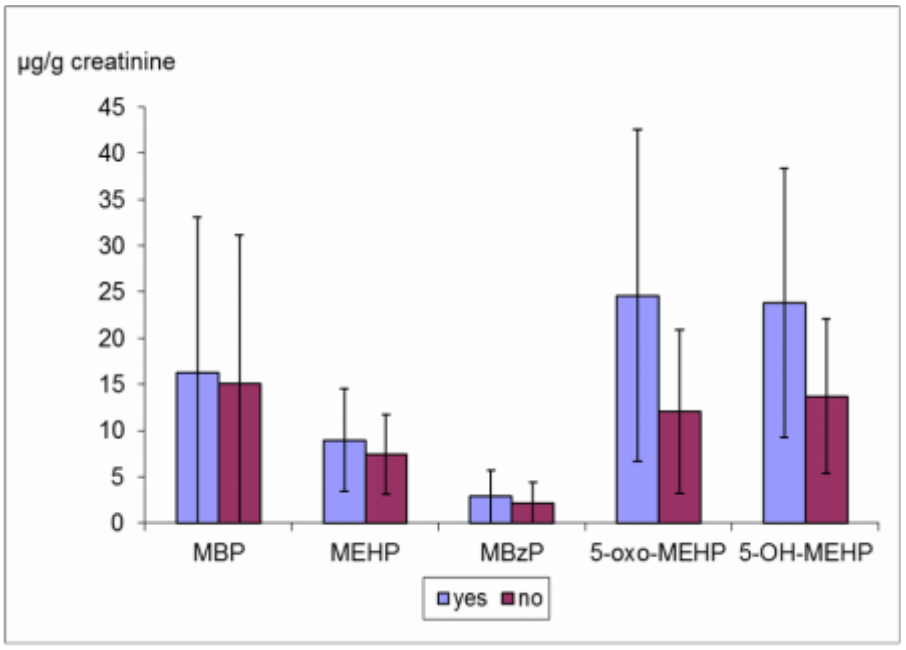
c) associations with possible identified risks (phthalates in water and food)

Other risk factors, in addition to water consumption habits, are heating food in plastic containers in the microwave oven, use of cosmetics and personal care products and also the car used.

The majority of subjects (64%) drink water both from the distribution network and bottled water, while only 3 subjects drink water only from the public system. As a result, interpretation of metabolites concentration of phthalates in urine for a given type of water consumption is difficult to achieve.

As regards the day before the exposure, the responses to the questionnaire on water consumption in the last 24 hours that preceeded the collection of urine sample showed that subjects kept the same behavior regarding water consumption as the one declared in the questionnaire filled up at the beginning of the study (9 only bottled water, 3 network water, 13 mixed water).

In the case of the use of microwave ovens for heating food in plastic pots, results showed a statistically significant difference for two of the five metabolites, i.e. 5-oxo-MEHp ($p=0.029$), and 5-OH-MEHP ($p=0.039$), people with this habit having much higher concentrations in urine than those who do not have this behavior (Figure 32).



metabolite	p
MBP	0,860
MEHP	0,452
MBzP	0,509
5-oxo-MEHP	**0,029**
5-OH-MEHP	**0,039**

Figure 32. Average values and Student’s t-test between concentrations of urinary biomarkers depending on the use of microwave oven for heating food.

Unlike the recently published data (Cerna et al., 2014) who found that the only factor leading to significant differences in the concentration of phthalates in urine is the use of personal care products, in our study, conducted on a small number of subjects, we did not find such an association.

d) daily intake

Setting the daily intake is crucial in determining the dose-effect relationship and actually anticipate the possible side effects on health status.

In this subchapter we proposed to calculate the daily intake of phthalates based on all routes of exposure (ingestion, inhalation, dermal contact), based on urinary metabolite concentrations measured in all subjects in our studies (68 subjects).

In order to calculate DEHP intake, concentrations of the three analyzed metabolites were summed up based on the urinary excretion factors.

The results were compared with values found in other biomonitoring studies and with international benchmarks (EFSA).

The main method for cancer risk assessment, for a certain level of exposure in case of mixtures containing chemicals, is the calculation of the hazard index for

phthalates by summing up the daily intake for each phthalate related to the reference values (Christensen et al, 2014; Frederiksen et al., 2014). The method is specifically recommended for groups of chemical substances toxicologically similar for which data on the dose-response relationship exist (EPA, 2004).

Results and discussions

Table 40 presents the summary statistics of the daily intake of three phthalates for the group of 25 volunteer subjects who participated in the biomonitoring study in relation to multiple sources of exposure to phthalates.

	DBP µg/kg_body/day	DEHP µg/kg_ body/day	BBzP µg/kg_ body/day
median	0.398	2.195	0.068
average	0.495	2.611	0.067
minimum	0.022	0.615	0.0002
maximum	1.464	5.926	0.267
refrence value	10	50	500

Table 40. Summary statistics of the calculated daily intake and reference values.

The average daily intake for each of the three compounds was higher in women than in men, BBzP being the only statistically significantly higher (p=0.0001).

The European Food Safety Authority (EFSA) recommends as reference daily intake the following values: 10 µg/kg_body/day for DBP, 50 µg/kg_body/day for DEHP and 500 µg/kg_body/day for BBzP. Based on these benchmarks, none of the subjects reached these values, moreover all values were an order of magnitude, even three orders of magnitude (for BBzP) lower.

The values obtained for the daily intake are slightly higher than those obtained in France by Martine et al., 2013 who calculated the daily intake based only on their concentrations in drinking water, food and ambient air.

In practice, due to the lack of information on the mode of action and pharmaco-kinetics, the requirement for similarity from a toxicological point of view is limited to the similarity of target organ (endocrine system). A method taken into account in order to associate exposure to phthalates with possible health effects is the hazard index.

The average value of hazard indices was 0.102 (values between 0.017 and 0.249), an order of magnitude lower than value 1, above which it is possible that

toxicological effects of the investigated substances may occur, suggesting that subjects' health is not endangered at the moment in terms of exposure to phthalates.

Since the hazard indices calculated for the group of 25 subjects who filled up a complex questionnaire on possible sources of exposure to phthalates focusing on water consumption were low, we calculated the hazard indices for the group of subjects who participated in another study performed by our team, whose urine samples were used to develop the analytical method.

For this group of subjects the calculated hazard indices ranged between 0.015 and 1.123, the average being 0.122, well below the value 1 considered critical in expectation of effects related to exposure to phthalates. It should be pointed out that one of the subjects whose urine sample was analyzed in the pilot study had the hazard index over the value 1 (1.123) meaning that there is a potential risk of antiandrogenic effect, the daily intake for DBP being higher than the reference value (11.23 µg/kg_body/day).

Comparing the median values of hazard coefficients (for each individual compound) of the two groups of urine samples analyzed, Figure 34 shows that in both cases DEHP is the one that contributes with more than half to the hazard index. We have to remember that DEHP, besides its endocrine disruptor effect, is the only phthalate classified as possibly carcinogenic to humans, and especially that there are people, even in our study, for whom exposure to DEHP is significant and with carcinogenic risk, its contribution being similar in both groups.

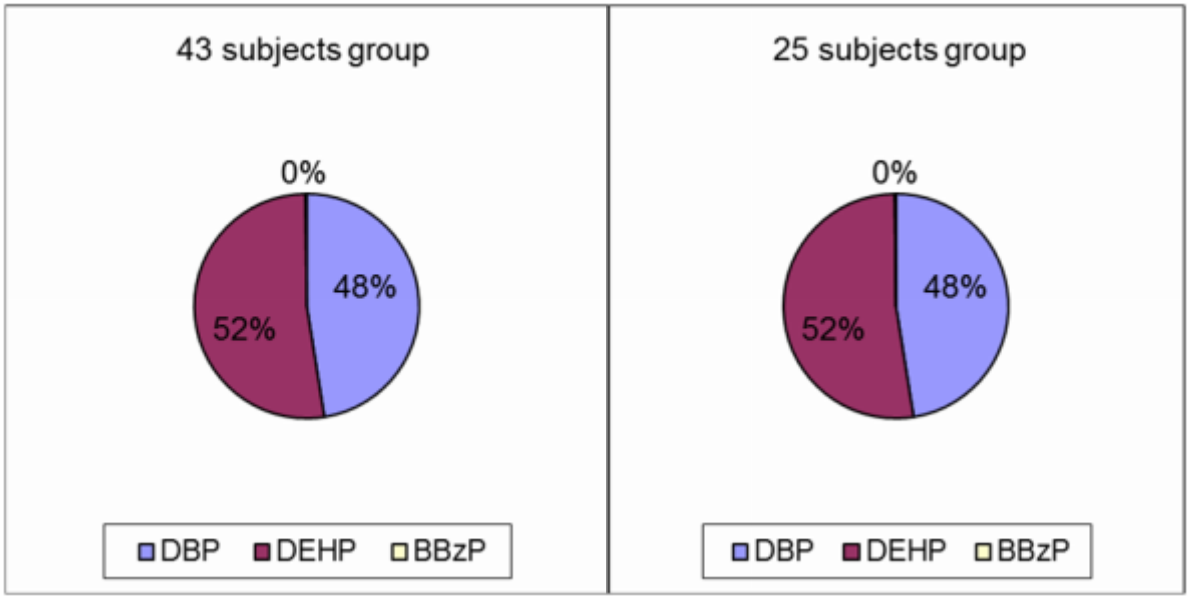


Figure 34. Contribution of determined phthalates to the calculated hazard index

4.5. Behavior analysis. Risk factors, ways to reduce and control

In the earlier stages of our study, by processing the concentrations of urinary metabolites for subjects who completed the questionnaire of exposure to phthalates, a single significant risk factor resulted regarding lifestyle, namely heating food in plastic containers in the microwave oven.

In this chapter we proposed a behavioral analysis regarding also other factors that may represent, especially cumulatively, a significant potential risk factor in exposure to phthalates. Another objective is proposing methods to reduce and control the behavioral risk factors identified.

Materials and method

Each of the questions in the questionnaire related to the possible exposure to phthalates was coded and given a number of points depending on the response version referring to the quantitative aspects and frequency of use. A total score was calculated after scoring each answer. Partial scores were also calculated by dividing the responses from the questionnaire into three categories: water consumption (both bottled and from the public network), food consumption (packaged and heated in plastic or disposable containers) and lifestyle (use of cosmetics, use of car, home interior finishing).

We set an ideal score of 15 points related to a low exposure to phthalates by adopting a lifestyle that limits the interaction with products containing phthalates.

Results and discussions

Behavioral scoring performed as indicated above shows, for the investigated subjects, an average score of 6.4 for water consumption and a similar score (17.96 and 17.28, respectively) for food and lifestyle. The average total score for the group of interviewed subjects was 41.64.

Compared to the ideal score, the higher deviations (Figure 35) in terms of exposure to phthalates ocurr in the case of lifestyle and food.

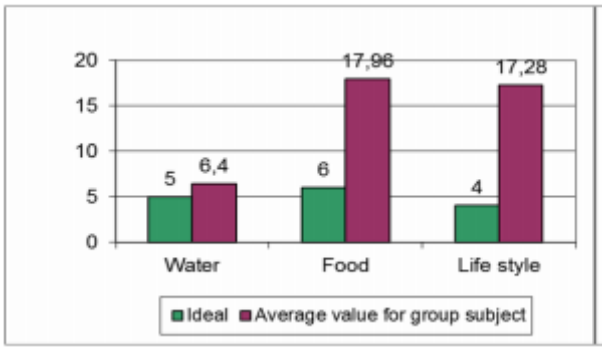


Figure 35. Average score on categories

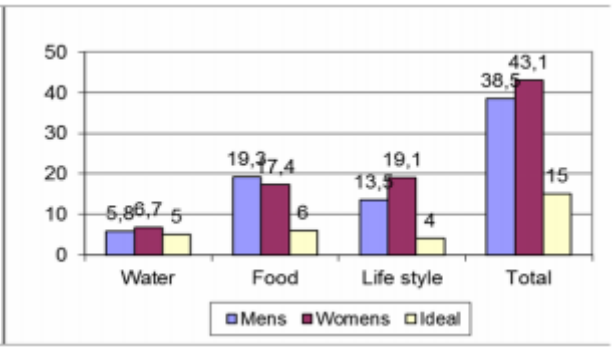


Figure 36. Average score on sexes

The separate calculation of the score for men and women (Figure 36) showed differences between these two categories for the average score. For food and water these values are close, but for lifestyle there is a significant difference in the case of women, the Student's t-test value being 0.005 in this case (a value below 0.05 indicating a correlation between the two analyzed categories).

Compared to the calculated ideal score we observe that in terms of water drinking habits the average values obtained for males (5.8) are closer to ideal, while for both women and men scores for food and lifestyle are much higher (food 19.3 and 13.5, respectively 17.4 and 19.1) than those proposed for a healthy life in terms of exposure to phthalates.

Next we divided the total scores for both men and women into three categories, multiples of the proposed ideal score (Table 42): low-risk in exposure to phthalates (15-30), medium risk (31-45, over two-fold the ideal value) and high risk respectively (over 45, more than three-fold the ideal score value).

Most of the men (62.5%) were placed in the average score category, of up to three-fold higher than the ideal score. Also,about half of thewomen (47%) were placed in the high risk category, more than three-fold higher (≥ 46). Only two women (8% of the investigated group) and one man (4% of the investigated group) had the behavioral score calculated to be up to two-fold higher than the ideal score (calculated score ranged between 15-30). To conclude we can say that women have a behavior with risk in relation to phthalate exposure .

The behavioral analysis performed in our study indicates a risk factor that is not reflected in the statistical analysis of urinary metabolites concentrations with respect to behavior, namely the use of cosmetics.

Methods to reduce and control risks associated with exposure to phthalates

The questionnaire-based investigated group is highly educated personnel that works in the same institution and is in contact with the research and their results from different segments of the anthropogenic effect upon the environment and health. However, their behavioral practices are with risk in exposure to phthalates. Having educational background and field-specific knowledge, such a group may be a target group for which we can create an efficient model of change.

In order to create a model of change as a result of our studies we propose several phases:

- Selection of the target group to promote and generate behavioral change (group of 25 subjects);
- Creating prerequisites for change - information, presentation of results, removal of available means that allow unhealthy behaviors for the group (proper keeping of bottled water and excluding heating food in plastic or catering type containers in the microwave oven);
- Implementation of an efficient demonstrative program for adopting innovation or behavioral changes (proper keeping of bottled water, e.g. demonstrative measuring of the concentration of phthalates after a period of time);
- Using the power of example that has been successful in other communities for the dispersion of behavioral changes - training the group for further dissemination (in the family and to close friends).

Conditions for change are created by increasing knowledge and awareness of the group about the benefits of new practices to improve the quality of life.

A behavior based on intensive use of plastics is a risk factor in the generation of this type of waste, which is hardly biodegradable and leads to environmental contamination with chemicals (as explained in chapter 4.3.2., concentrations of exclusively synthetic compounds were measured in surface water sources). According to literature, higher concentrations of phthalates were found in groundwater near landfills (Mihovec-Grdić et al., 2002).

Huge amounts of plastics are reaching landfills, most of which are positioned proximity to localities and have faulty management. In addition, landfills represent only a concealment of the problem, not its solution, the delayed natural degradation

processes, infiltrations and huge quantities of waste will extend the pollution indefinitely.

Changing the regulations based on the accumulation of new knowledge requires both an economical and social assessment, as it must assess the financial costs of change as accurately as possible. In addition to the economic costs, the administration and policy makers also intervene. If this is missing, it is unlikely that educational measures to protect health areto succeed.

The first and most important step in the management of plastic waste and their effects on the environment, the ecosystem and human health as a whole is to limit the production of such waste. And this can only be accomplished by changing the related human behaviors.

Changing attitudes / behaviors

Focus on changing attitudes/behaviors as the primary means of behavioral change must have as precondition raising the awareness regarding the benefits of the proposed new practices in improving the quality of life. Research suggests that attitudinal and behavioral changes are often effective when certain conditions are created to support the desired behavior.

Social education is not sufficient to promote behavior. Population responsiveness must be encouraged by creating optimal conditions for learning new behaviors, providing resources and positive initiatives for their adoption and support to the social system that sustains them.

Implementing a healthy behavior change program requires the transmission of knowledge and new skill sets to potential subjects who adopt it. Role models must be provided for them.

Resistance to change

Some people are more conservative and not easily adopt innovations, others are less resistant to innovations and adopt them easily. A person is more willing to learn new practices from short therm contacts and innovations extend easier in cohesive groups than in those with weaker social ties.

A program of public information should be directed primarily toward these two sectors with high risk factors (diet and lifestyle). In terms of product and industry,

withdrawing or replacing disposable packaging and returning to the returnable packaging (glass) or biodegradable (paper) calls into question both economic issues, as this industry is highly developed and profitable, and policy issues. On the other hand reducing plastic packaging would bring at least two major benefits for the environment: improving waste management as they are not recycled, and secondarily contamination of water sources from landfills which includes plastic waste, their breakdown leading to a leachate including phthalates with possible contamination of groundwater (old landfills).

Part of the intervention program should be to list on the cosmetics label (as listed for parabens), at least DEHP which is identified as possibly carcinogenic; use of quality materials with minimum content of phthalates (scientific studies showed phthalates transfer from packaging into bottled water in much lower concentrations than what we found in our study, which means that there technical is a posbility to use better materials). The population must be instructed to read the label and choose between a packaged product in plastic material (or plasticized) and a biodegradable (paper) or neutral (glass).

5. Conclusions

Phthalates have a special place in today’s toxicology because of their almost ubiquitous presence in the environment and their multiple and severe effects on the human body, including their possible carcinogenicity.

The study was divided in four parts:

- developing proper analysis methods of phthalates from liquids and biological matrices;
- researching the transfer of phthalates from content packaging (bottled water, food);
- determining phthalate content from public drinking water supply systems;
- measuring biomarkers for phthalate exposure in humans (metabolites from urine) and calculating the integrated human exposure to three phthalic acid diesters.

The development of new analytical methods that can identify low concentrations of these compounds is a current requirement for analytical methods that address various matrices in which phthalates are present.

Due to the particularities of each analytical instrument and especially those of the analyst, gas chromatographic analysis methods can be improved by slightly varying the conditions (temperature program of the oven, injection port temperature, flow rate of the carrier gas, injection volume and type).

Performance evaluation of analytical methods, such as limit of determination, precision, accuracy, recovery and uncertainty from various matrices more or less complex (water/urine) shows that they are suitable for their intended purpose, meeting the requirements set.

The phthalate analysis method from liquid samples (bottled water and packaging used in catering) developed in this study, allowed us to identify them in different experimental conditions of measurement.

Storage conditions, the contact duration between the contents and the package, temperature (high values) as well as pH (alkaline) along with the type of packaging, the amount of plasticizer used in the packaging manufacturing and filling process, are contributing factors to the amount of compounds found in water.

Phthalate concentration measured after opening the bottle exceeded the US-EPA regulations for one of the samples, while after 25 days of storage at room temperature concentrations exceeded the WHO recommendations for three of the five samples of bottled water analyzed.

None of the groundwater sources analyzed in the study (from the rural area) were contaminated with phthalates, unlike the investigated surface water source that was contaminated with these compounds, most likely because of the PET waste containers.

Although in low concentrations, but increasing during the water treatment process, DBP was identified at entrance in the distribution network, but only at the surface water source treatment plant.

Two compounds (DBP and DIBP) were identified in the water distribution networks. They were in varying concentrations in all the investigated supply systems, the highest concentrations were measured in the networks exclusively using HDPE pipeline.

Just like the usual parameters used for water quality monitoring, the presence of phthalates is influenced by the materials used in the manufacturing of the distribution pipes (if case of phthalates - HDPE), which can release phthalates in drinking water, having a contribution to the daily intake of these types of compounds known to have health effects.

Study results suggest that, in case of the surface water source and the samples analyzed at the end of the technological process, temperature and UV radiation play a role in the biodegradation of DiBP.

Reversly, the increase in phthalate levels resulted to be influenced by water stagnation in the distribution network and by a slightly alkaline pH. These are favoring factors for phthalatemigration to drinking water from pipes, reflected in the increasing rates of the identified compounds.

Biomonitoring of the phthalate exposure using the method for the determination of metabolites developed in this study allowed the identification of all the five metabolites analyzed in the urine samples. The biomonitoring was performed on a group of subjects for whom, according to the questionnaires, specific features regarding water consumption, frequency of use of personal care products, water and food storage/heating and living environment could be identified.

Concentration levels of the analyzed metabolites in urinein this study are much higher than the values obtained for the >20 years old population from the US, however, compared to the concentration levels from the European countries, MBzP values are lower, but DEHP values are slightly higher.

Statistically significant differences in the concentration levels of metabolites in urine have been found between subjects of the opposite sex.

Based on a preset ideal score, the analysis of subjects' behavior showed that women have a higher risk factor to phthalate exposure than men, probably due to the use of cosmetic products.

For overweight women, the exceeded average concentration index indicates that there are significant differences in the urinary excretion of metabolites.

Based on the information given by the questionnaires, heating food in plastic dishes in the microwave made the difference between metabolites concentration levels in urine, this being the only statistically significant difference in terms of life style for subjects that have this eating behavior.

Because of the specific metabolic features, the analysis of a single urine sample doesn't allow the determination in time and space of the highest phthalate exposure moment, and therefore metabolite concentration levels in urine, as there is an inherent uncertainty associated that may represent a limitation in the cumulative risk assessment.

Based on the metabolites found in the urinary samples, the daily intake of phthalates has been calculated. DEHP had the highest average value, followed by DBP and BBzP, the last one being significantly higher in women than men ($p=0.0001$). All calculated values were significantly lower than the daily intake reference, leading to hazard indices below 1.

Monitoring and controlling the drinking water quality with results corresponding to current legislation does not exclude the presence of other compounds that may have adverse health effects. In terms of contamination with phthalates, Romanian and European legislation should have at least references to DEHP as a possible carcinogen.

Despite the resistance to changes, human behavior modification on avoiding risk factors in exposure to phthalates, along with technical measures regarding the composition of materials that come in contact with water and food, but also limiting other sources of exposure to phthalates are strategies that can significantly contribute to limiting the health effects of many substances and mixtures to which humans are exposed.

6. Originality and innovative contributions

- The study shows, for the first time in Romania, the transfer possibility of phthalic acid diesters from packaging to food/water by identifying the concentrations of these compounds, sometimes significant, and relating them to the storage and consumption conditions;

- It is the first study in Romania that identifies phthalates from public water supply systems, starting with the source (surface water/ground water) to the treatment plant and the distribution pipeline network to the consumer;

- It is the first study in Romania that highlights the presence of phthalates in distribution networks exclusively made of high density polyethylene pipes. According to our knowledge, there are no published data regarding the phthalate content and transfer from high density polyethylene into water;

- There are no published data about phthalate metabolite concentration levels in urine in Romania;

- This is the first study in Romania that calculates the daily intake of phthalates based on biomarker concentration levels in urine (multiple exposure sources and pathways);

- This is the first study in Romania that shows the contribution of lifestyle behaviors on significantly influencing exposure to phthalates.

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