Foodstuff Storage and Phthalates: Effects on Human Health

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Summary

Concern about how exposure to environmental contaminants may interfere with human health and its consequences is in constant rise. Phthalates, unlike other environmental contaminants, are not persistent or bioaccumulative but its broad use makes them ubiquitous in today's world. Although concentrations of phthalates in different products vary it has been proven that the most significant source of human exposure to phthalates is packaging material of various food products. Other contamination can occur also during production process, transportation and storage time in different packaging and wrapping materials. People could be exposed to phthalates through food, water, cosmetics and personal care products, toys and environment depending on dietary habits as well as personal lifestyle. Large number of studies has proved effects of phthalate exposure on human male reproductive development, sperm quality, risk factors for cancer, allergies, asthma and obesity, while impact on cardiovascular health remains unclarified. This article gives a quick, brief and insightful overview on numerous literature data of phthalate exposure in humans due to food storage and their impact on health.

Key words

phthalates, phthalates migration, food packaging, food, human health

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Introduction

Phthalates are synthetically produced compounds generated by esterification of phthalic acid and different alcohols (Ventrice et al., 2013; Karačonji et al., 2017). They are divided into two categories depending on the type of alcohol: high molecular weight phthalates and low molecular weight phthalates (Ventrice et al., 2013) (Table 1.). The chemical structure of phthalate consists of benzene ring and two ester functional groups (Katsikantami et al., 2016). The reaction of phthalate formation occurs in two steps. First step is rapid and irreversible monoester formation while the second step is catalyzed reversible reaction of monoester conversion to diester (Mariana et al., 2016). Phthalates, besides being widely used in various products for general use, are usually added in plastics for improvement of softness, flexibility and extensibility (Ventrice et al., 2013; Karačonji et al., 2017). Due to their wide range of usage, they are ubiquitous in today's world and can be found in toys, cosmetics, cleaning products and food packaging. The fact that they are not chemically bounded to the product sets the risk of their possible release into the air, water, food and environment. This indicates that the most significant source of human exposure could be packaging material. Food can come in contact with phthalates in the production process, transportation and storage in plastic containers, wrappers, tubes, pipes or some other systems containing plastics (Karačonji et al., 2017; Dong et al., 2017). They are mainly used in PVC (polyvinyl chloride) materials, but could be found also in other polymers like polypropylene (PP), polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET) and even cellulose-based polymer materials. There is even the possibility that the plastic from the food packaging could release phthalates into food no matter from what polymer it's made off. Various phthalates are used for different products so accordingly to that, one can say that exposures to phthalates also varies, depending on diet pattern and product use. Cosmetics and personal care products may contain several types of phthalates such as diethyl phthalate (DEP), di-n-butyl phthalate (DBP) and di-isobutyl phthalate (DIBP), while in food and pharmaceutical packaging the most common are DEP, DIBP and di(2-ethylhexhyl) phthalate (DEHP) (Johns et al., 2015). DEHP is mostly widespread phthalate in products (Karačonji et al., 2017). In 2012 France was the first EU country which banned the use of plastic containing DEHP in pediatric, neonatology and maternity wards (Katsikantami et al., 2016).

The aim of this article is to review the literature linking exposure to phalates as a consequence of normal consumer activities mainly dietary food intake, domestic and industrial food preparation, regular household maintenance, and health. The relevant literature was collected searching databases PubMed, Science Direct, Web of Science, Scopus and Google Scholar, particularly in the area of environmental pollutants and health. Cutoff point for publication inclusion was year 2010 because from that year we can observe significant increase in number of research publications, up to around 590 publications per year, indicating the importance of the possible role of the phalates in human health. Only two publications regarding single research in Croatia were form earlier years.

Metabolic pathways and toxicity of phthalates

Upon intake, phthalates are relatively quickly metabolized and extracted from organism in urine and feces. Their hydrolisation and conjugation pathways differ by each phthalate. Mainly it includes phthalate diester transformation into more bioactive monoester metabolite by different lipases and esterases in the intestinal

Table 1. Phthalates classification

Molecular weight, solubility	Name	Abbreviation	Toxicity	Major metabolite	Abbreviation metabolite	Usage	Excretion
Low, water soluble	Dimethyl phthalate	DMP	Toxic for reproduction (category 1B)	Mono-ethyl phthalate	MEP	Solvent	After hydrolization (without oxidation)
	Diethyl phthalate	DEP		Mono benzyl phthalate	MBzP, MBeP	Solvent, fixative	
	Benzylbutyl phthalate	BBP				Plasticizer, solvent	
	Dibutyl phthalate	DBP	Toxic for reproduction (category 1B[1])	Mono-butyl phthalate	MBP	Solvent, plasticizer (PVC, rubber), fixative	Mostly after hydrolization
	Diisobutyl phthalate	DIBP	Toxic for reproduction (category 1B)	Mono-isobutyl phthalate	MiBP	-	
	Di-2-ethyl-hexyl phthalate	DEHP	Toxic for reproduction (category 1B)	Mono (2-ethylhexyl) phthalate	MEHP, MEHHP, MEOHP	Plasticizer for PVC	Like hydrolizes monoesters and oxidation metabolites
Heigh, less water soluble	Diisononyl phthalate	DINP	No	Mono iso-nonylphthalate	MiNP	Plasticizer for PVC	
	Diisodecyl phthalate	DIDP	No	Monocarboxyisononyl phthalate	MCNP, MCINP	Plasticizer for PVC	
	Di-n-octyl phthalate	DOP		Mono (2-ethyl 5-hydroxyhexyl) phthalate	MEHHP	Plasticizer for PVC	

[1] First category: Category 1B – hazardous compounds believed to have carcinogen effect for humans, based on animal experiments (according to EC regulation No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (DOI: 2004R0726 - v.7 of 05.06.2013)

epithelium, liver, blood and other tissues. Those monoester phthalate metabolites are then biotransformed in two different ways; to glucuronide-conjugated monoesters which are excreted by urine, or they form more hydrophilic secondary oxidized metabolites prior to glucuronidation, which are then less bioactive. Monoester phthalate metabolites could also just directly excrete in urine, without any further biotransformation and are called unconjugated free monoesters (Johns et al., 2015). The phthalate exposure pathway for humans is ingestion, inhalation, dermal and intravenous exposure, as well as crossing the placental barrier. In children, exposure to phthalates is twice as high, due to their lower body mass as well as handmouth activities. Low molecular weight phthalates DEP, DBP and benzylbutyl phthalate (BBP) are volatile and are present as solvents and fixatives in some products. They are more likely to be inhaled or absorbed through skin, while DEHP is predominantly used as plasticizer and it's more likely to be ingested. Phthalate toxicity is associated with endocrine disruption, allergies development, asthma, fertility problems and cancer. As endocrine disruptors (ECDs) phthalates imitate estrogen and androgen, which are naturally occurring hormones, producing overstimulation. They bind to receptors and in that way they block endogenous hormones from binding. Free monoesters cause toxicity while parent compounds and glucuronidated metabolites are non-toxic (Katsikantami et al., 2016). When monitoring and evaluating the exposure of particular contaminant and its effect on human health, epidemiological studies play an important role. Johns (2015) in his epidemiological study showed greater temporal stability of short chained rather than long chained phthalate metabolites in urine samples. Although the measurement of phthalate concentration was made in various matrixes like blood, serum, semen, breast milk, sweat and saliva, urine measurement has shown to be the best biomarker for epidemiological studies and currently the most accepted method.

As phthalates are hormone mimickers they are associated with testicular dysgenesis syndrome (TDS) in men, as well as reduced testosterone levels and sperm quality. Hypothyroidism in mothers during pregnancy is in the correlation with urine phthalate metabolites and represents the risk for mental retardation and fetal neurodevelopment. Phthalate exposure in mothers induces differences in DNA methylation which leads to gene expression modifications that affects health significantly further in life. Also, the exposure in the prenatal period leads to a later problem in childhood, having tendency to delinquent and aggressive behavior and learning disabilities. Recent studies highlighted the association of phthalate exposure in prenatal phase with neurobehavioral disorders, syndromes and lower IQ in children aged 6 to 10 years. Infants can also be exposed to phthalates through breastfeeding, because phthalate concentration in breast milk was positively associated with the diet of mothers as well as their water consumption. In newborns such exposures are connected with low birth weight and length of infants, preterm childbirth, head circumference and femur length. Exposure to phthalates due to inhalation during childhood is associated with allergy development, obesity, ant-androgen effect, delay of growth and puberty, as well as changes in systolic blood pressure (Katsikantami et al., 2016). Recent studies indicate phthalates as cardiovascular disruptors what connects them again to diseases like obesity, atherosclerosis, lipid metabolism and blood pressure problems like hypertension. Nutrition Examination Survey (NHANES) identified some phthalate monoesters as predictors of abdominal obesity. These studies have demonstrated a certain association, but due to the short half-lives of phthalates additional studies are required. Phthalates also interfere the insulin signaling and thus increase the oxidative stress (Muscogiuri and Colao, 2017). After banning specific phthalates in USA, studies confirmed the reduction of phthalate metabolites in urine samples from 2001 to 2010. This decline does not have to be strictly related to legislation but to potential changes in the products composition as well as the habits of their use. It is also necessary to emphasize the occurrence of exposure differences in different populations. Children are mostly exposed to phthalates through toys. Since exposure to one of the most common phthalates such as DEHP decreases, exposure to another phthalate increases, which is used as its substitute. As it has been previously said, DEHP is one of the most common plasticizer (Mariana et al., 2016) and is rapidly metabolized after exposure to mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono (2-ethyl-5-oxohexyl) phthalate (MEOHP). About 75% of those metabolites are removed from the body within two days. Those DEHP monoesters could be associated with interruption of reproductive hormone and also testicular toxicity. Fong et al. (2015) investigated DEHP metabolites in urine samples in flexible-PVC workers. The measurements discovered higher concentrations for measured metabolites than those measured in previous studies conducted on general population pointing to the possibility that occupational exposure could be much higher than general population exposure through diet or lifestyle. Fong's study also found connection between urinary DEHP metabolites and serum estradiol (E_2) levels and the E_2:T (estradiol : testosterone) ratio but couldn't be certain is this effect induced by DEHP exposure or some other congener. He also points out the concern about possible long-term DEHP exposure in workplace that can cause disruptions of the reproductive hormone circulation. Recommendations would be to use protective equipment when working with PVC products as well as more frequent ventilation of work areas (Fong et al., 2015). Another study however mentions phthalates as one of the well-studied chemicals that perturb androgen synthesis and mentions the different phthalate effects regarding to different species with similar effects of receptor antagonists over these species (Skakkebeak et al., 2015).

Phthalates migration from food packaging to food

Phthalates can migrate from plastic products to the environment under influence of different temperatures and pH and thus contaminate food and water (Cao, 2010; Katsikantami et al., 2016). Back in 1976, Tomita, Nakamura and Yagi found a close correlation between phtalic acid ester (PAE) residues and specific foodstuffs. The study also revealed high levels of DNBP and DEHP in human blood samples of healthy individuals and the levels rose significantly after meals. Bradley (2012) made a FSA project C01048 (Food Standard Agency's project which aimed to establish the source of phthalates in foodstuffs) with the report for determination of 17 phthalate diesters, 9 phthalate monoesters, phtalic acid and total phthalate in foods with establishing methodology to distinguish phthalate source. The report counts twenty food groups and their 119 categories. Accordingly, to his study, DEHP was the most prevalent phthalate followed by phthalic acid. Two most abundant diesters were DBP and DEHP reflected also as monoesters. DiNP was also determined in some food samples. The report specifically confirms and concludes that phthalates could be found in food due to the migration from food contact materials as a source, and food is the

most important and responsible human exposure pathway. That is because the food is often wrapped in materials that contain phthalates or its just contaminated during food processing (Muscogiuri and Colao, 2017). The highest phthalate concentrations are found in fatty foods like fish, meat, vegetable oils and diaries, due to the long lipophilic chains (Karačonji et al, 2017). Shen et al. (2015) established the connection between type of a diet and food preparation with phthalate exposure among Shangai school-age children. He related it also with intake of different pharmaceutical and herbal supplements which have phthalate plasticizers in their coatings. Karačonji et al. (2017) overviewed the literature on phthalate occurrence in alcoholic beverages and concluded that no matter how the contamination happened, alcoholic beverages may contain relatively high phthalate concentration, DBP and DEHP specifically. Dong et al. (2017) studied the relationship of phthalate exposure and food contact materials pointing out the significance of risk assessment linked to flexible plastic containers. He revealed that there is a connection between phthalate exposure and consumption of food from plastic containers during the previous three days. It was two-year study on 2 140 male Shanghai adults in who's urine samples DEHP metabolites were most frequently detected. Similarly, Fong et al. (2015) pointed out connection of food contamination with DEHP through contact with PVC during processing and packaging of food. According to another study (Sakhi et al., 2014) phthalate concentrations measured in common Norwegian foods and beverages showed the presence in all tested products pointing out diisononyl phthalate (DiNP) (84%) which is one of the DEHP substitutes, and dicyclohexyl phthalate (DCHP) (11%) as the most common ones. Meat products and grains were the main contributors for dietary phthalate exposure in the adult population of Norway. Also, their mean dietary exposure to phthalates was lower than tolerable daily intake (TDI) values established by European Food Safety Agency (EFSA). TDI for DnBP, BBzP, DEHP has been specified to be 10, 500, 50 μg/kg body weight (bw)/day, and for DiNP and DiDP a group TDI was set to be 150 μg/kg bw/day, respectively. Other cross-sectional study (Shen et al., 2015) revealed that diet is an important source of phthalate exposure among school-aged children in Shanghai. Study showed positive association between monobutyl phthalate (MBP) and seafood and negative association between (MBP) and dried fruits and vegetables while egg consumption showed negative association for all DEHP metabolites.

Animal testing on phthalate exposure showed quite concerning results. DEHP, which is widely used phthalate, has shown to be rodent liver carcinogen, and together with DBP, BBzP and their metabolites (MEHP, MBP and MBzP) have teratogenic effect in animals. As the use of phthalates in food packaging material reduces, there are still some materials that contain those plasticizers so the migration into food is the major path of exposure. Because of many health concerns, some countries like Denmark, back in 1989 banned the use of DEHP in milk industry, milk tubing specifically, and Norway followed that example replacing DEHP with other plasticizers. By observing migration levels of di–2-ethylhexyl adipate (DEHA) from PVC film packaging plasticized material into cheese it was concluded that higher levels of DEHA concentrations are achieved with longer contact time while there was no increase in concentration levels with higher temperature. Also, there were found higher DEHA concentrations in cheese with higher fat content (Cao, 2010). Jarošová and Bogdanovičová (2015) investigated phthalate migration (DBP and DEHP specifically) in individual samples and packaging during 28 days of storage. The study showed rising tendency in phthalate concentrations and values ranged from ≤0.2 mg/kg to 11.11 mg/kg for DBP and 0.58 mg/kg 28.20 mg/kg for DEHP. Schecter et al. (2013) investigated phthalates concentration in various US food products in state of New York and found DEHP and DCHP to be the most common ones (74% and 6%). Pork had the highest estimated mean concentration (mean 300 ng/g and maximum 1 158 ng/g). Estimated intakes for adult population were ranging from: 0.004 μg/kg/day for DMP to 0.0673 μg/kg/day for DEHP. Jeddi et al. (2014) in their work were concerned about the migration of phthalates from plastic bottles into the water. Study examined migration of DBP, BBP and DEHP in bottled water under various storage conditions. Evaluation showed that bottled water was safe for children consumption. The conclusion of the study was that temperature increase and duration of storage affect phthalate migration but DEHP concentrations were specified as very low and don't exceed US Environmental Protection Agency maximum concentration limit (MCL) of 26.83%. Estimated child phthalate intake was generally in the safe range and decreased with increasing age. The study in Iran (Rastkari et al., 2017) investigated storage time, temperature and bottle type on the phthalate migration from different packaging materials into acidic contents. They found the highest concentrations of DEP and DEHP in PET and HDPE (high-density polyethylene). The analysis before and after specific storage conditions showed increase in DEP, DEHP and DBP concentrations in acidic liquids but do not represent a relevant ingestion source due to low concentration detected. They concluded that concentrations of phthalates found in bottled liquid samples surely depend on all three examined factors. Another review (Bhunia et al., 2013) on migration of different chemical compounds from packaging materials during microwave, conventional heating treatment and storage showed how migration of phthalates was higher when food remained in contact with the packaging material for longer period of time. PVC was found not to be suitable for microwave treatment. Swelling of polymers was noted as an important factor accelerating the migration of additives under increased temperature and durations. Again, they explained how more studies should be performed to evaluate the microwave influence on the phthalate migration level from various polymer packaging. Study on phthalate migration from plastic containers to soft drinks and mineral water (Bošnir et al., 2006) showed that migration depends on drink pH (lower pH higher phthalate migration, specifically). They found DMP as the highest rate of migration in soft drinks (53.51 to 92.73%) and DBP and DEHP in the highest rate of migration from plastic containers to mineral water (56.04 and 43.42% respectively). Bošnir et al. (2003) also investigated migration of phthalates as plastic softeners from different plastic products into model solutions and its possible adverse effect on human health. The highest pooled level of phthalates released was for plastic toys where migration happened most rapidly (66.2 mg/kg) followed by food containers (37.6 mg/kg) and consumer goods such as plastic plates and cutlery (27.4 mg/kg). However, the concentrations of migrated phthalates were found not to be harmful for human health nor even for a longer period of time. Berger et al. (2017) in their study investigated personal care products associated with higher exposure to phthalates indicating that it may be relevant in adolescent girls who frequently use personal care products in their important and critical reproductive period. Higher urinary concentrations of MEP, MBP, MP and PP were found in girls who reported recent use of specific makeup products. Katsikantami et al. (2016) in their work mentioned that exposure to phthalates by

skin contact can be also through clothing, increasing phthalate contamination with wearing time and points out that it's possible to remove low molecular weight (LMW) phthalates by laundering.

Determination of phthalates in food

Sample preparation for determination of different phthalates depends on type of the sample. Sample preparation of food samples involves extraction and clean-up procedure, separation step and finally the detection of the specific analyte. Extraction and clean-up procedures are the most critical steps in the preparation process. Extraction is often based on multiple liquid-liquid extraction, with single or mixture of solvents. Water is removed with sodium sulfate following by evaporation and concentration step under nitrogen flow. Mixture of solvents could be used for dissolving the residue before clean-up procedure. Clean-up procedure is necessary for isolating fat from phthalate and it is often done by size-exclusion chromatography (SEC). The extracts are then injected onto packed column with specific packaging material suitable for specific components of interest. The column elutes with specific solvents and the cleaned extract is captured finally in fractions. Detailed sample preparation due to specific phthalate has been previously showed in Cao, 2010 study. Weinzl et al. (2009) give comprehensive instructions in "Methods for the determination of phthalates in food" as a survey conducted among European laboratories in food control.

Phthalates and human health

There are many advances in phthalate biomarker measurements that are promising in improvement of epidemiological data. Long-term exposure to phthalates might lead to molecular and epigenetic imprinting which has lifelong effect on steroid hormone performance (Benjamin et al., 2017). Studies also confirm concerns about DEHP potential carcinogenicity although the epidemiological evidence in humans is incomplete. They connect DEHP with induced tumorigenesis and even reduced chemosensitivity (Chou et al., 2017). Benjamin et al. (2017) also point how phthalates are especially linked to liver, skin and gastrointestinal cancers generally and breast cancer in woman. They also point how phthalates in rodents are connected with skeletal malformations, while in humans there are only two recent studies which demonstrates strong association of urinary metabolites of DEHP, DEP, MBP, mono(3-carboxypropyl) phthalate (MCPP) and MBzP with reduced femur neck and total hip bone mineral density in postmenopausal woman and MEP, MBP, MiBP, MBzP with osteoporosis in femur neck and spine. A large number of studies show association of high weight phthalates and their monoesters with allergies and asthma. Exposure to phthalates is also recognized as major contributor to obesity what could be independent of diet and physical activity (Benjamin et al., 2017). Other study indicated how obese children could reduce phthalate intake by replacing unhealthy with healthy diet (less packaged and processed food) (Correia-Sá et al., 2018). Campbell et al. (2018) made a realistic exposure sub-model DEHP physiologically- based pharmacokinetic model for human adults. It included the relations of body weight to energy intake and the DEHP intake, describing the positive association between BMI and DEHP. Praveena et al. (2018) reveal in their review how continuous exposure to phthalates in humans lead to liver dysfunction due to inhibition of liver detoxifying enzymes. They suggest stem cell therapy which *in vivo* studies proved to lead again to liver functioning properly. Stem cells could induce hepatic lineage on injured liver with promoting hepatocyte proliferation. Some studies gave positive associations for MCiOP, MCNP and MiNP with systolic blood pressure and due to that a possible risk for hypertension (Katsikantami et al., 2016). Another study also observed that association, with a conclusion how DEHP exposure might be associated with increased systolic blood pressure and that DMP and DBEP exposure could be associated with increased total cholesterol concentration in serum. Despite the evidences of possible mechanism why is that so stay unclear (Zhang et al., 2018). Connections are also noted for phthalate exposure and speed up or delayed puberty in girls and boys due to inverse associations with pubic hair growth in boys and positive associations with breast onset (Katsikantami et al., 2016). The findings also showed how DEHP metabolites in girls with early puberty were much higher than in the control group (Hashemipour et al., 2018). Exposure throughout embryonic development could show adverse health effects later in life, although they may not be present immediately after birth (Shu et al., 2018). Zhao et al. (2017) demonstrated how exposure to phthalates, same as exposure to passive smoke or heavy metals are risk factors for abortion (Zhao et al., 2017). Newborns who are held in intensive care hospital units could be exposed to DEHP through the use of special medical devices (Hauser and Calafat, 2005).

According to Agarwal et al. (2015) 30 million of men around the globe are infertile, highly rated in Africa and Eastern Europe. It has become a global health issue. Men are found to be solely responsible for 20-30% infertility couple cases, contributing to half of overall cases (Agarwal et al., 2015). The evaluation of male infertility should consist from much more than a simple semen analysis. It should also consider physical examination, comprehensive history anamnesis including environmental pressures and relevant endocrine and genetic investigation (Esteves et al., 2012a). Jurewicz et al. (2013) for the first time in the same study assessed semen quality parameters, sperm chromatin structure, sperm aneuploidy and level of reproductive hormones in adult men of Lodz in Poland referring to the relationship between levels of phthalate metabolites and numerical chromosomal sperm aberrations.

According to biological monitoring of four phthalates (DEP, DEHP, DnBP, BBzP) (Tranfo et al., 2012) the analysis of five metabolites in urine samples confirmed the assumption that exposure to phthalates from different sources, have influence on the fertility of both genders In other study (Tranfo et al., 2013) examined the profile of urinary phthalate metabolites in Italy and concluded that occupational exposure occurs in different geographical areas, revealing similar patterns in USA, China and Mexico (Tranfo et al., 2013).

Study on urinary phthalate concentrations in mothers and children in rural and urban areas of Ireland as well as information about their lifestyle and environmental conditions confirmed widespread distribution of phthalate exposure in that country. Results showed higher concentrations in families with lower achievements and also in those exposed to PVC, junk food and personal care products. All seven tested phthalate biomarkers were found in Irish children samples while five of them were detected in all mothers. The most common phthalate biomarkers found in children were MiBP and MEP, while in mothers it was the opposite (firstly MEP and then MiBP). Also, children from urban areas had higher MEOHP concentrations compared to children from rural areas. As for other exposure to phthalates, all DEHP metabolites (MEOHP, MEHHP and MEHP) in children were related with chewing gums, while MiBP in children were connected to PVC exposure at home, while MEP concentrations in mothers were linked to personal care products

and dietary habits. Higher MnBP concentrations in mothers were linked with plastic gloves (Cullen et al., 2017). Cao (2010) highlights that PVC gloves sterilized with alcohol increase phthalate migration from gloves into foods. Mendiola et al. (2012) suggest association of DEHP exposure with some changes in circulating levels of male sex steroid hormones, what is in coherence with its anti-androgenic effect.

Meeker et al. in study from 2010 suggest that urinary DEHP metabolite concentrations found in general US population may be in connection with altered steroid hormone levels and aromatase activity. Acrosin activity was measured in specific studies due to the fact that it's a potential marker in clinical studies for semen quality. Inhibition in acrosin activity also prevents fertilization. Pan et al. (2015) in their study discuss the correlation between 14 phthalate metabolites in urine samples from Chinese adult men and their specific hormone levels as well as semen parameters. They detected phthalate metabolites in most of the urine samples (>98%) except MBzP and MOP, emphasizing MBP as the highest concentration found (78.7ng/ml) and MOP as the lowest, even below limit of quantification (LOQ) so they didn't include it in further analysis. Correlations were found between all phthalate metabolites (Pan et al., 2015). Also, another cohort study (Liu et al., 2012) measured six phthalate monoesters (MMP, MEP, MBP, MBzP, MEHP and MEOHP) in urine samples of 150 individuals of Chongqiung (China) reproductive institute and detected MEP and MEOHP in all urine samples while MMP, MBP and MEHP in 99.3%, 97.3% and 98.7% urine samples. The lowest detection rate was for MBzP (44.7% of samples) probably due to the lower exposure to BzBP. They also stated that they couldn't find significant differences in the mean phthalate levels for all subjects suggesting that phthalate levels in urine depend on time of exposure and elimination kinetics. Another cross-section study (Han et al., 2014) examined MBP, MEP, MEHP, MBzP, phtalic acid (PA) and total PA in urine samples of men from the same clinic in Chongquing and concluded that exposure to environmental level of phthalate have weak or no adverse effects on the men reproduction health. They mention that differences between the results from other studies could be due to variations in the studied population, sample size (232 men), age (20-40 years old) and of course lifestyle and daily habits. Mendiola et al. (2011) studied the relationship between eleven phthalate metabolites and reproductive hormones including follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), inhibin B, estradiol and sex hormone-binding globulin (SHBG). Study of 425 men in SFF (study for future families) were examined in five US cities from 1999 to 2005 and revealed minor alterations for free testosterone markers due to DEHP exposure. Mediolas study from 2012 showed DEHP exposure to be robustly associated with male sex steroid hormones in both, fertile and infertile men. Chang et al. (2017) examined eleven phthalate metabolites in urine and semen of 253 sub fertile and fertile men from Reproductive Medicine Center in National Cheng Kung University Hospital in southern Taiwan. It was the first human study which confirmed the hypothesis how exposure to phthalate, especially DEHP, decreases sperm production by inhibiting insulin-like factor 3 (INSL3) expressions and testosterone levels in adult men. Study showed significant correlation of seminal concentration of MEP, MEHHP and MECPP with those detected in urine. Study (Axelsson et al., 2015) on relationship of DEHP metabolite levels in urine and several semen quality parameters (progressive motility and high DNA stainability (HDS) which is a marker for sperm immaturity, specifically) was examined in 314 young Swedish adolescents aged 17-20. The study showed negative association of DEHP metabolite levels and sperm motility and maturation that indicated that exposure to DEHP in adulthood has negative impact on male fertility. Another study (Pant et al., 2008) investigated the correlation between phthalate esters (DEP, DEHP, DBP, DMP, DOP) and sperm mitochondrial status, reactive oxygen species (ROS), lipid peroxidation (LPO), sperm chromatin structure assay (SCSA), as well as sperm quality. Among 300 participants from Locknow Obstetrics and Gynecology Department Chhatrapati Shahuji aged 21-40 who were divided in fertile and infertile groups, infertile men showed higher levels of pollutants in semen than fertile men. Also, urban population had higher values of phthalate esters than rural population. Study established adverse effects indicating that phthalates might be contributing to deterioration in semen quality. Meeker et al. (2009) detected MEP in 100% urine samples of 425 subfertile men, aged 18 to 55, in Boston infertility clinic (MGH). MBP and MBzP were detected in 97% and 94% urine samples, and MEHP in 83% of samples. MEHP showed inverse relationship with circulating steroid hormone levels in adult men. Other cross-sectional study (Lenters et al., 2018) on 602 male partners of pregnant women during antenatal care visit in Greenland, Poland and Ukraine discovered 15 contaminants, including DEHP and DiNP in more than 70% blood samples. The inverse association between DiNP and testosterone, and between DEHP and neutral α-glucosidase was also noticed which was proof that some contaminants including phthalates have adverse effect on reproductive health in men.

Case-control study conducted in Infertility Clinic in Taiwan (Chang et al., 2015) discovered higher concentration of MnBP, MEHP and mono-2-ethyl-5-carboxypentyl phthalate in infertile than in fertile men, lower INSL3 level in infertile men as well as inverse association between some phthalate metabolites and specific semen quality parameters (between MMP, MiBP, MEHP and serum of total testosterone (TT), between MiBP, MBzP MEHP and serum of free testosterone (fT), between MMP, MEHP and TT:LH ratio, and between MMP, MiBP MnBP, MBzP, MEHP and free androgen index (FAI)). This study also showed negative association of phthalate metabolites (MBzP and MEHP) with decrease in INSL3 in serum. Wang et al. (2015) also confirmed higher phthalates levels in infertile men than in fertile, with concentration variations depending on age (older men had higher concentrations of phthalate metabolites). Pant et al. (2010) observed *in vivo* and *in vitro* relationship between some phthalate metabolites (DEHP, DBP) and sperm motility resulting in significant correlation. Another study confirmed association of phthalate exposure and specific sperm parameters like MEHP and decreased testosterone levels, semen volume and total sperm count. DiNP metabolites have shown an androgenic effect by decrease in serum testosterone and SHBG (Specht et al., 2014). Lenterset al. (2018) gave one of the largest studies to date and identified inverse association between DiNP and circulating testosterone.

The effect of phthalate exposure to human health is still unclarified. Laboratory experiments on rodents showed significant impact but those low doses of exposure on humans are very hard to determine. However, there is a large body of evidence on effects of phthalate exposure on human male reproductive development as well as sperm quality, risk factors for cancer, allergies, asthma and obesity, while impact on cardiovascular health remains unexplained (Cullen et al., 2017). Esteves et al. (2012b) pointed out new reference values for semen parameters reported by World Health

Organization (WHO) which are lower than previously reported ones. They suggested how technological progress today should bring us more robust and cost-effective clinically useful tests to assess the fertilizing potential of semen samples.

Conclusion

Even as short insight on phthalates exposure in today's modern world gives us a clearer picture on harmfulness of these chemicals. It has been proven before how phthalates migrate from various packaging material and specific storage conditions into food and food products. It can certainly be said how our lifestyle and daily habits are reflecting on our health status. A number of studies are claiming that exposure to phthalates has adverse effects on human health, most commonly associating it with reproductive health in men. This is a growing problem today due to more frequent infertility in men. Since phthalates are ubiquitous in today's environment we can say that they are practically inevitable. Regulatory agencies try to monitore and reduce contaminants like phthalates banning their usage but sometimes it's just not enough. We recommend a change in lifestyle and dietary habits to reduce contact and therefore phthalate exposure on individual basis.

References

Agarwal A., Mulgund A., Hamada A., Chyatte M.R. (2015). A unique view on male infertility around the globe. Reprod Biol Endocrinol 13:37. doi: 10.1186/s12958-015-0032-1

Axelsson J., Rylander L., Rignell-Hydbom A., Jönsson B.A.G., Lindh C.H., Giwercman A. (2015). Phthalate exposure and reproductive parameters in young men from the general Swedish population. Environ Int 85: 54–60. doi: 10.1016/j.envint.2015.07.005

Benjamin S., Masai E., Kamimura N., Takahashi K., Anderson R.C., Abdul P. (2017). Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. J Hazard Mater. 340: 360–383. doi: 10.1016/j.jhazmat.2017.06.036

Berger K.P., Kogut K.R., Bradman A., She J., Gavin Q., Zahedi R., Parra K.L., Harley K.G. (2018). Personal care product use as a predictor of urinary concentrations of certain phthalates, parabens, and phenols in the HERMOSA study. J Expo Sci Environ Epidemiol 1–12. doi: 10.1038/s41370-017-0003-z

Bhunia K., Sablani S.S., Tang J., Rasco B. (2013). Migration of chemical compounds from packaging polymers during microwave, conventional heat treatment, and storage. Compr Rev Food Sci Food Saf. 12 (5): 523–545. doi: 10.1111/1541-4337.12028

Bošnir J., Puntarić D., Galić A., Škes I., Dijanić T., Klarić M., Grgić M., Čurković M., Šmit, Z. (2007). Migration of phthalates from plastic containers into soft drinks and mineral water. Food Technol Biotechnol. 45 (1): 91–95

Bošnir J., Puntarić D., Škes I., Klarić M., Šimić S., Zorić I. (2003). Migration of phthalates from plastic products to model solutions. Coll Antropol 27 Suppl 1: 23–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12955888

Bradley E.L. (2012). Determination of phthalates in food and establishing methodology to distinguish their source. Food Standards Agency (FSA), London, 1-305.

Campbell J. L., Yoon M., Ward P.L., Fromme H., Kessler W., Philips M.B., Anderson W.A., Clewell H.J. III, Longnecker M.P. (2018). Excretion of Di-2-ethylhexyl phthalate (DEHP) metabolites in urine is related to body mass index because of higher energy intake in the overweight and obese. Environ Int. 113: 91–99. doi: 10.1016/j.envint.2018.01.023

Cao X.L. (2010). Phthalate Esters in Foods: Sources, Occurrence, and Analytical Methods. Compr Rev Food Sci Food Saf. 9 (1): 21–43. doi: 10.1111/j.1541-4337.2009.00093.x

Chang W.H., Li S.S., Wu M.H., Pan H.A., Lee C.C. (2015). Phthalates might interfere with testicular function by reducing testosterone and insulin-like factor 3 levels. Hum Reprod. 30 (11): 2658–2670. doi: 10.1093/humrep/dev225

Chang W.H., Wu M.H., Pan H.A., Guo P.L., Lee C.C. (2017). Semen quality and insulin-like factor 3: Associations with urinary and seminal levels of phthalate metabolites in adult males. Chemosphere 173: 594–602. doi: 10.1016/j.chemosphere.2017.01.056

Chou C.K., Yang Y-T., Yang H-C., Liang S-S., Wang T-N., Kuo P-L., Wang H-M.D., Tsai E-T., Chiu C-C. (2017). The Impact of Di(2-ethylhexyl)phthalate on Cancer Progression. Arch Immunol Ther Exp. 0(0): 1–15. doi: 10.1007/s00005-017-0494-2

Correia-Sá L., Kasper-Sonnenberg M., Pälmke C., Schütze A., Norberto S., Calhao C., Domingues V.F., Koch H.M. (2018). Obesity or diet? Levels and determinants of phthalate body burden - A case study on Portuguese children. Int J Hyg Environ Health: 0–1. doi: 10.1016/j.ijheh.2018.02.001

Cullen E., Evans D., Griffin C., Burke P., Mannion R., Burns D., Flanagan A., Kellegher A., Schoeters G., Govarts E., Biot P., Casteleyn L., Castaño A., Kolossa-Gehring M., Esteban M., Schwedler G., Koch H.M., Angerer J., Knudsen L.E., Joas R., Joas A., Dumez B., Sepai O., Exley K., Aerts D. (2017). Urinary phthalate concentrations in mothers and their children in Ireland: Results of the DEMOCOPHES human biomonitoring study. Int J Environ Res Public Health. 14 (12). doi: 10.3390/ijerph14121456

Dong R.H., Zhang H., Zhang M.R., Chen J.S., Wu M., Li S.G., Chen B. (2017). Association between Phthalate Exposure and the Use of Plastic Containers in Shanghai Adults. Biomed Environ Sci 30 (10): 727–736. doi: 10.3967/bes2017.098

Esteves S.C., Hamada A., Kondray V., Pitchika A., Agarwal A. (2012a). What every gynecologist should know about male infertility: An update. Arch Gynecol Obstet. 286 (1): 217–229. doi: 10.1007/s00404-012-2274-x

Esteves S.C., Zini A., Aziz N., Alvarez J.G., Sabanegh E.S., Agarwal A. (2012b). Critical appraisal of world health organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men. Urology 79 (1): 16–22. doi: 10.1016/j.urology.2011.08.003

Fong J.P., Lee F.J., Lu I.S., Uang S.N., Lee C.C. (2015). Relationship between urinary concentrations of di(2-ethylhexyl) phthalate (DEHP) metabolites and reproductive hormones in polyvinyl chloride production workers. Occup Environ Med. 72 (5): 346–353. doi: 10.1136/oemed-2014-102532

Guo Y., Wang L., Kannan K. (2013). Effect of storage time and temperature on levels of phthalate metabolites and bisphenol A in urine. Adv Environ Res. 2 (1): 9–17. doi: 0.12989/aer.2013.2.1.009

Han X., Cui Z., Zhou N., Ma M., Li L., Li Y., Lin H., Ao L., Shu W., Liu J., Cao J. (2014). Urinary phthalate metabolites and male reproductive function parameters in Chongqing general population, China. Int J Hyg Environ Health 217(2–3):271–8. doi: 10.1016/j.ijheh.2013.06.006

Hashemipour M., Kelishadi R., Amin M.M., Ebrahim K. (2018). Is there any association between phthalate exposure and precocious puberty in girls?. Environ Sci Pollut Res. doi: 10.1007/s11356-018-1567-4

Hauser R. and Calafat A.M. (2005). Phthalates and human health. Occup Environ Med. 62(11): 806–818. doi: 10.1136/oem.2004.017590

Jarošová A., Bogdanovičová S. (2015). Phthalate migration from packaging materials into food. Potravinarstvo. 9(1):275–9. doi: 10.5219/479

Jeddi M.Z., Rastkari N., Ahmadkhaniha R., Yunesian M. (2014). Concentrations of phthalates in bottled water under common storage conditions: Do they pose a health risk to children? Food Res Int 69: 256–265. doi: 10.1016/j.foodres.2014.11.057

Johns L.E., Cooper G.S., Galizia A., Meeker J.D. (2015). Exposure assessment issues in epidemiology studies of phthalates. Environ Int 85: 27–39. doi: 10.1016/j.envint.2015.08.005

JRC. Methods for the determination of phthalates in food. Outcome of a survey conducted among European food control laboratories. JRC Scientific and Technical Reports. (2009). 1-50 p. Available from: %5C%5CRobsrv-05%5Creference manager%5CArticles%5C15953.pdf

Jurewicz J., Radwan M., Sobala W., Ligocka D., Radwan P., Bochenek M., Hawuła W., Jakubowski L., Hanke W. (2013). Human urinary phthalate metabolites level and main semen parameters, sperm chromatin structure, sperm aneuploidy and reproductive hormones. Reprod Toxicol 42: 232–241. doi: 10.1016/j.reprotox.2013.10.001

Karačonji I.B., Jurica S.A., Lasić D., Jurica K. (2017). Facts about phthalate toxicity in humans and their occurrence in alcoholic beverages. Arh Hig Rada Toksikol 68 (2): 81–92 doi: 10.1515/aiht-2017-68-2951

Katsikantami I., Sifakis S., Tzatzarakis M.N., Vakonaki E., Kalantzi O.I., Tsatsakis A.M., Rizos A.K. (2016). A global assessment of phthalates burden and related links to health effects. Environ Int 97: 212–236. doi: 10.1016/j.envint.2016.09.013

Lenters V., Portengen L., Smit L.A.M., Jönsson B.A.G., Giwercman A., Rylander L., Lindh C.H., Spanò M., Pedersen H.S., Ludwicki J.K., Chumak L., Piersma A.H., Toft G., Bonde J.P., Heederik D., Vermeulen R. (2015). Phthalates, perfluoroalkyl acids, metals and organochlorines and reproductive function: A multipollutant assessment in Greenlandic, Polish and Ukrainian men. Occup Environ Med. 72 (6): 385–393. doi: 10.1136/oemed-2014-102264

Liu L., Bao H., Liu F., Zhang J., Shen H. (2012). Phthalates exposure of Chinese reproductive age couples and its effect on male semen quality, a primary study. Environ Int 42 (1): 78–83. doi: 10.1016/j.envint.2011.04.005

Mariana M., Feiteiro J., Verde I., Cairrao E. (2016). The effects of phthalates in the cardiovascular and reproductive systems: A review. Environ Int 94: 758–776. doi: 10.1016/j.envint.2016.07.004

Meeker J.D., Calafat A.M., Hauser R. (2010). With Decreased Steroid Hormone Levels in Adult Men. 30 (3): 287–297. doi: 10.2164/jandrol.108.006403.Urinary

Mendiola J., Jørgensen N., Andersson A.M., Calafat A.M., Silva M.J., Redmon J.B., Sparks A., Drobnis E. Z., Wang C., Liu F., Swan S. H. (2011). Associations between urinary metabolites of di(2-ethylhexyl) phthalate and reproductive hormones in fertile men. Int J Androl. 34(4 PART 1): 369–378. doi: 10.1111/j.1365-2605.2010.01095.x

Mendiola J., Meeker J.D., Jørgensen N., Andersson A.M., Liu F., Calafat A.M., Redmon J.B., Drobnis E.Z., Sparks A.E., Wang C., Hauser R., Swan S.H. (2012). Urinary concentrations of Di(2-ethylhexyl) phthalate metabolites and serum reproductive hormones: Pooled analysis of fertile and infertile men. J Androl. 33 (3): 488–498. doi: 10.2164/jandrol.111.013557

Muscogiuri G., Colao A. (2017). Phtalates: new cardiovascular health disruptors? Arch Toxicol. 91 (3): 1513–1517. doi: 10.1007/s00204-016-1780-1

The European Parlament and of the Council of European Union. Directive 2000/53/Ec of the European Parliament and of the Council of 18 September2000 on End-of-life Vehicles, Brussels, Official Journal of the European Communities, (L 269) 1-15. doi: 2004R0726 - v.7 of 05.06.2013

Pan Y., Jing J., Dong F., Yao Q., Zhang W., Zhang H., Zhang H., Yao B., Dai, J. (2015). Association between phthalate metabolites and biomarkers of reproductive function in 1066 Chinese men of reproductive age. J Hazard Mater 300: 729–736. doi: 10.1016/j.jhazmat.2015.08.011

Pant N., Pant A.B., Shukla M., Mathur N., Gupta Y.K., Saxena D.K. (2011). Environmental and experimental exposure of phthalate esters: The toxicological consequence on human sperm. Hum Exp Toxicol. 30 (6): 507–514. doi: 10.1177/0960327110374205

Pant N., Shukla M., Kumar Patel D., Shukla Y., Mathur N., Kumar Gupta Y., Saxena D.K. (2008). Correlation of phthalate exposures with semen quality. Toxicol Appl Pharmacol. 231 (1): 112–116. doi: 10.1016/j.taap.2008.04.001

Praveena S. M., Teh S.W., Rajendran R.K., Kannan N., Lin C-C., Abdullah R., Kumar S. (2018). Recent updates on phthalate exposure and human health: a special focus on liver toxicity and stem cell regeneration. Environ Sci Pollut Res. 1–21. doi: 10.1007/s11356-018-1652-8

Rastkari N., Jeddi M.Z., Yunesian M., Ahmadkhaniha R. (2017). The effect of storage time, temperature and type of packaging on the release of phthalate esters into packed acidic liquids. Food Technol Biotechnol. 55 (4): 562–569. doi: 10.17113/ftb.55.04.17.5128

Sakhi A.K., Lillegaard I.T.L., Voorspoels S., Carlsen M.H., Løken E.B., Brantsæter A.L., Haugen M., Meltzer H.M., Thomsen C. (2014). Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ Int 73: 259–269. doi: 10.1016/j.envint.2014.08.005

Schecter A., Lorber M., Guo Y., Wu Q., Yun S.H., Kannan K., Hommel M., Imran N., Hynan L.S., Cheng D., Colacino J.A., Birnbaum L.S. (2013). Phthalate concentrations and dietary exposure from food purchased in New York state. Environ Health Perspect. 121 (4): 473–479. doi: 10.1289/ehp.1206367

Shen Q., Shi H., Zhang Y., Cao Y. (2015). Dietary intake and phthalates body burden in boys and girls. Arch Public Heal 73 (1): 5. doi: 10.1186/2049-3258-73-5

Shu H., Jönsson B.A.G., Gennings C., Svensson Å., Nånberg E., Lindh C.H., Knutz M., Takaro T.K., Bornehag C-G. (2018). Temporal trends of phthalate exposures during 2007–2010 in Swedish pregnant women. J Expo Sci Environ Epidemiol. doi: 10.1038/s41370-018-0020-6

Skakkebaek N.E., Rajpert-De Meyts E., Buck Louis G.M., Toppari J., Andersson A-M., Eisenberg M.L., Jensen T.K., Jørgensen N., Swan S.H., Sapra K.J., Ziebe S., Priskorn L., Juul A. (2015). Male Reproductive Disorders and Fertility Trends: Influences of Environment and Genetic Susceptibility. Physiol Rev 96 (1) :55–97. doi: 10.1152/physrev.00017.2015

Specht I.O., Toft G., Hougaard K.S., Lindh C.H., Lenters V., Jönsson B.A.G., Heederik D., Giwercman A., Bonde J.P.E. (2014). Associations between serum phthalates and biomarkers of reproductive function in 589 adult men. Environ Int 66: 146–156. doi: 10.1016/j.envint.2014.02.002

Tomita I., Nakamura Y., Yagi Y. (1977). Phthalic acid esters in various foodstuffs and biological materials. Ecotoxicol Environ Saf. 1 (2): 275–287. doi: 10.1016/0147-6513(77)90043-4

The European Parliament (2011). European Regulatory classification criteria, hazzard communication elements, Prevention.

Tranfo G., Caporossi L., Paci E., Aragona C., Romanzi D., De Carolis C., De Rosa M., Capanna S., Papaleo B., Pera A. (2012). Urinary phthalate monoesters concentration in couples with infertility problems. Toxicol Lett 213 (1): 15–20. doi: 10.1016/j.toxlet.2011.11.033

Tranfo G., Papaleo B., Caporossi L., Capanna S., De Rosa M., Pigini D., Corsetti F., Paci E. (2013). Urinary metabolite concentrations of phthalate metabolites in Central Italy healthy volunteers determined by a validated HPLC/MS/MS analytical method. Int J Hyg Environ Health 216 (4): 481–485. doi: 10.1016/j.ijheh.2012.11.003

Ventrice P., Ventrice D., Russo E., De Sarro G. (2013). Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity. Environ Toxicol Pharmacol 36 (1): 88–96. doi: 10.1016/j.etap.2013.03.014

Wang S.Y., Wang Y., Xie F.Q., Li Y.X., Wan X.L., Ma W.W., Wang D-C., Wu Y-H. (2015). Analysis of PAEs in semen of infertile men. Int J Occup Environ Health. 21 (1): 40–48. doi: 10.1179/2049396714Y.0000000088

Zhang S., Shen Y-x., Li L., Fan T-T., Wang Y., Wei N. (2018). Phthalate exposure and high blood pressure in adults: a cross-sectional study in China. Environ Sci Pollut Res.

Zhao R., Wu Y., Zhao F., Lv Y., Huang D., Wei J., Ruan C., Huang M., Deng J. (2017). The risk of missed abortion associated with the levels of tobacco, heavy metals and phthalate in hair of pregnant woman. Medicine. 96(51). doi: 10.1097/MD.0000000000009388