

Phthalates: Toxicology and Food Safety – a Review

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Abstract

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Phthalates are organic substances used mainly as plasticisers in the manufacture of plastics. They are ubiquitous in the environment. Although tests in rodents have demonstrated numerous negative effects of phthalates, it is still unclear whether the exposure to phthalates may also damage human health. This paper describes phthalate toxicity and toxicokinetics, explains the mechanisms of phthalate action, and outlines the issues relating to the presence of phthalates in foods.

Keywords: di(2-ethylhexyl)phthalate; dibutyl phthalate; peroxisome proliferators; reproductive toxicity

Phthalic acid esters, often also called phthalates, are organic substances frequently used in many industries. They are usually colourless or slightly yellowish oily and odourless liquids only very slightly soluble in water. Phthalates are much more readily soluble in organic solvents, and the longer their side chain, the higher their liposolubility and the boiling point. Phthalates have a broad variety of uses. They are used as the so-called plasticisers, i.e. substances that improve mechanical properties of plastic materials, mainly PVC. They are also used in the manufacture of floorings, children's toys, and are added to printing inks and to perfumes and nail varnishes. Such a broad range of applications in the industry brings about the problem of an extensive phthalates – caused contamination of the environment where they are nowadays ubiquitous. This is because phthalates are not chemically bound in plastics in any way, and they are relatively easily released from them to the external environment (water,

air, soil, food, etc.). People as well as animals can be exposed to these compounds through ingestion, inhalation or dermal exposure, and iatrogenic exposure to phthalates from blood bags, injection syringes, intravenous cannulas and catheters, and from plastic parts of dialysers is also a possibility (VELÍŠEK 1999; ČERNÁ 2000; LOVEKAMP-SWAN & DAVIS 2003). HAUSER *et al.* (2004) described abnormally high concentrations of monobutyl phthalate (MBP) in urine of a patient treated for ulcerative colitis. As part of his therapy, the patient received Asacol tablets for three months. The tablets with controlled release were coated with a polymethacrylate film, which was the probable source of phthalates.

Toxicity

Acute toxicity of phthalates is very low. Low molecular phthalates, e.g. diethyl phthalate (DEP), may cause irritation of the skin, conjunctiva, and

the mucous membrane of the oral and nasal cavities in animals. However, similar reactions are not as a rule encountered in humans (API 2001; GÓMEZ-HENS & AGUILAR-CABALLOS 2003).

Much more important are subchronic and chronic toxic effects of phthalates. Most important, numerous experiments in rodents have shown adverse effects of phthalates on the reproductive system and on the intrauterine development of foetuses. EMA *et al.* (1995, 1996) monitored the effects of monobenzyl phthalate (MBzP) and monobutyl phthalate (MBP) administered to female rats during pregnancy. It follows from their studies that the exposure to phthalates at doses of about 500 mg/kg during pregnancy may cause in female rats an increase in the number of foetus resorptions and dead foetuses, lower weights of the offspring at birth and, last but not least, foetus malformations, e.g. cleft palate, atresia ani, and skeletal deformations.

Female rats exposed to di(2-ethylhexyl) phthalate (DEHP) at a dose of 2000 mg/kg also demonstrated a prolongation in their estrous cycles and anovulation as a result of a decrease in serum estradiol levels. The anovulation was related to the absence of the corpus luteum in the ovary and the occurrence of follicular cysts (LOVEKAMP-SWAN & DAVIS 2003).

Testicular lesions, hypospadias, cryptorchidism and other disorders in sexual organs of male rats were also found, which testify to antiandrogenic effects of some phthalates, particularly of dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP) (MOORE *et al.* 2001). Although there are no doubts about adverse effects of phthalates on the reproduction and development in rodents, the effects of phthalates on humans have not been satisfactorily clarified to date. The main reason is that only very few studies of the effects of phthalates on humans are available. COLÓN *et al.* (2000) monitored levels of certain phthalates in the blood serum of young Puerto Rican girls aged 6 months to 8 years with a premature breast development (thelarche). The authors demonstrated significantly higher phthalate levels in 68% of patients, which suggests possible negative effects of phthalates on the human reproduction and development.

Other negative effects of the exposure to phthalates may have in rodents are hepatotoxicity and carcinogenicity (DAVID *et al.* 1999; ELCOMBE *et al.* 2002). These effects have not, however, been described in other animal species or the human

yet. The authors reported hepatocyte proliferation, increased liver weight, and subsequent appearance of liver tumours in both sexes of rats and mice exposed to high doses of DEHP in food, i.e. at least 2500 mg/kg of food.

Toxicokinetics

In the case of human exposure to phthalates, phthalate diesters are relatively rapidly hydrolysed to their respective monoesters in the intestine by pancreatic or liver hydrolases (the first stage of phthalate biotransformation). The monoesters thus produced are bioactive molecules responsible for the adverse effects of phthalates. Monoesters are absorbed in the blood stream and then metabolised in liver. They are subject in a varying degree to hydroxylation and oxidation reactions that enhance water solubility of the products. Phthalate monoesters with a short side chain are oxidised to a lesser extent. The second stage of phthalate biotransformation is conjugation with glucuronic acid mediated by the enzyme UDP-glucuronyl transferase. The conjugation affects mainly monoesters and their oxidised metabolites with a long side chain because the conjugation facilitates the excretion of relatively lipophilic metabolites. Both the conjugated and the free (non-conjugated) phthalate metabolites are excreted in urine and partly also faeces (SILVA *et al.* 2003; KATO *et al.* 2004).

Diethyl phthalate (DEP) deviates in some respects from the above pattern. On the basis of their measurements, SILVA *et al.* (2003) assumed that DEP was absorbed unchanged from the intestine and was hydrolysed to monoethyl phthalate (MEP) in kidneys. Because MEP is relatively readily soluble in water, it need not be transported to liver for conjugation and is excreted in urine mainly in the non-conjugate form.

A mechanism similar to that described in the human takes place during phthalate metabolism in rodents. A relatively large number of metabolite types have been reported in urine of rats exposed to butyl benzyl phthalate (BBP), including phthalic acid, hippuric acid, and trace amounts of benzoic acid. Hippuric acid is a conjugate of toxic benzoic acid and glycine (NATIVELLE *et al.* 1999). The conjugation of phthalate metabolites with glucuronic acid takes place in mice only, it practically does not occur at all in rats (ALBRO 1986; NATIVELLE *et al.* 1999).

Mode of action

Some phthalic acid esters are well-known peroxisome proliferators (PPs). A very potent peroxisome proliferator is di(2-ethylhexyl) phthalate (DEHP), while dibutyl phthalate, butyl benzyl phthalate, and diisononyl phthalate (DBP, BBP, DINP) are somewhat less effective (VALLES *et al.* 2003; SEO *et al.* 2004). It should be stressed that adverse effects of diester phthalates are attributable to their monoesters produced when diesters are hydrolysed in the gastrointestinal tract (HURST & WAXMAN 2003; SILVA *et al.* 2003). Peroxisome proliferators are bound by PPAR α - PPAR δ , i.e. peroxisome proliferator-activated receptors, which they activate (MELNICK 2001).

A role in the process of hepatotoxicity and carcinogenesis is played by subtype PPAR α . The activated PPAR α and the retinoid X receptor (RXR) combine to form a heterodimer. The PPAR α -RXR complex produced is specifically bound to PPREs (peroxisome proliferator response elements) in the promoter region of genes that control peroxisome proliferation. The transcription of these genes is activated by the PPAR α -RXR complex bond. The consequence is an increase in DNA synthesis, hepatocyte proliferation, hepatomegaly, induction of peroxisome and microsomal enzymes, and suppression of hepatocyte apoptosis (MELNICK 2001). Peroxisome enzymes participate in the metabolism of fats by enhancing β -oxidation of fatty acids in tissues. As a result of the oxidation processes of fatty acids, reactive oxygen species (ROS) and large quantities of hydrogen peroxide are generated that might aggravate tissue damage (the so-called oxidative stress) (SEO *et al.* 2004).

Studies have demonstrated great interspecific differences in the sensitivity to the effects of peroxisome proliferators. While the majority of rodents (mice, rats) are highly sensitive to PPs effects, the sensitivity or, rather, the responsiveness of humans, guinea pigs, and some other species to PPs is very weak or nonexistent. The low sensitivity of humans is probably due to the lower expression of PPAR α in the human liver compared with the liver of mice or rats (MELNICK 2001; HURST & WAXMAN 2003).

Toxicity of phthalates to the reproductive system of female rats is probably due to the suppression of the aromatase enzyme, which transforms testosterone in the cells of the *stratum granulosum* of the ovary follicles to estradiol. At the same time,

an increased activity of enzymes participating in the breakdown of estradiol in the liver of females exposed to DEHP and DBP was confirmed (LOVEKAMP-SWAN & DAVIS 2003). LOVEKAMP-SWAN and DAVIS (2003) also assumed that the female reproductive system may be damaged as a result of processes related to the peroxisome proliferation mediated not only through PPAR α but also through PPAR γ .

The mode of action of the toxic effects of phthalates to the male reproductive system has not yet been satisfactorily explained. An impairment of testosterone metabolism in testes of adolescent male rats has been observed, which is probably due to a number of factors. KIM *et al.* (2004) reported that testicular impairment and tubular atrophy were especially aggravated by hormone regulation disturbances that cause a decrease in the production of testosterone in testes, by adverse effects of reactive oxygen species and by testicular cell apoptosis. The impairment of the male reproductive system due to the DEHP is also caused by alterations of the cytosolic phospholipase enzyme A₂ (cPLA₂) and of enzymes that metabolise the arachidonic acid (KIM *et al.* 2004).

The factor that is probably responsible not only for the reproductive toxicity of phthalates but also for their teratogenicity is the availability of zinc in the period of foetal development. Zinc is an essential element for embryonic and foetal development. It has been demonstrated that DEHP exposure activates metallothioneins in the liver of pregnant females. Metallothioneins in the liver of females retain zinc and prevent it from being carried by blood to foetuses. PETERS *et al.* (1997) monitored zinc levels in the liver and plasma of pregnant female mice and of their foetuses. The authors found that zinc levels were increased in the liver of pregnant females but decreased in foetuses. It needs to be added that these changes were observed not only in PPAR α -positive homozygotic mice but also in transgenic PPAR α -negative homozygotic mice in which the gene for the production of PPAR α receptor had been removed. This seems to suggest that toxicity of phthalates for the male reproductive system of rodents and their teratogenicity are not due to a cascade of events connected with peroxisome proliferation and mediated through PPAR α (although it cannot be ruled out that they work through some other subtype of PPAR receptors). BBP-related zinc metabolism disturbances in rats have been

investigated by URIU-ADAMS *et al.* (2001). It is interesting to note that, contrary to the results with mice, no reduction in zinc availability for rat fetuses was found, but higher levels of plasmatic and liver iron in pregnant females exposed to high doses of BBP were demonstrated. It is not, however, clear whether this iron metabolism disturbance is related in any way to the reproductive toxicity and teratogenicity.

Because intensive research into the effects of phthalates is still underway, it is not yet possible to make any conclusions about the possible negative effects of phthalate exposure on the human health. Although most of adverse effects of phthalates are probably linked to peroxisome proliferation mediated through the PPAR α receptors to which humans shows practically no response, some toxic effects of phthalates seem to be PPAR α -independent. In spite of that it needs to be emphasised that the exposure doses used in the experiments with rodents were usually many times higher than the environmental doses humans are exposed to.

Phthalates in foods

The intake of phthalates contained in food is the most significant source of exposure for humans. It has been established that the amount of phthalates found in foods or meals depends on the initial contamination of ingredients used in the production of the food, food production technologies, the period of storage (the time of contact with packaging materials), storage temperatures, ways of preparing dishes, the fat content in foods, and the type of packaging material used (VELÍŠEK 1999).

A factor which may significantly increase phthalate concentrations in animal tissues and, subsequently, in foods is their fat content. JAROŠOVÁ *et al.* (1999) studied the distribution and accumulation of phthalates in the tissues of pigs and broiler chickens to which high doses of DEHP and DBP had been administered orally. The authors demonstrated that phthalates were distributed primarily to tissues with the high fat contents (subcutaneous fat and muscle tissue of pigs, mesenteric fat and skin of chickens), where they are also accumulated. The lipophilic character of phthalates was also demonstrated by measurements of phthalate concentrations in water, milk and dairy products (SHARMAN *et al.* 1994; PROKŮPKOVÁ *et al.* 2002). While the concentrations of DEHP in water sam-

ples varied from 0.49 $\mu\text{g/l}$ (deionised water) to 9.78 $\mu\text{g/l}$ (mineral water in glass bottles with metal caps and PVC seals) (PROKŮPKOVÁ *et al.* 2002), SHARMAN *et al.* (1994) reported total phthalate amounts between 0.06 and 0.32 mg/kg (of which DEHP < 0.01–0.09 mg/kg) in pooled milk samples and 19.00 mg/kg in cream samples (DEHP max. 2.70 mg/kg). The absolutely maximum concentration was found in cheese (114 mg/kg total phthalates, 17 mg/kg DEHP). High concentrations of phthalates in some Japanese retail packed lunches were demonstrated in 1999 by TSUMURA *et al.* (2001a, b). The source of phthalates were disposable PVC gloves worn in the preparation of packed lunches as a protection against the spreading of diarrhoeal diseases caused by *E. coli*. The amount of phthalates released to the dishes further increased if the gloves had been disinfected with ethanol. Because DEHP levels found in foods and dishes repeatedly exceeded the tolerated daily intake (TDI), the Japanese government banned the use of disposable PVC gloves for the handling of foods and dishes. After the ban in 2001, phthalate levels in foods averaged 4% of the values found in foods before the ban (TSUMURA *et al.* 2002). Phthalate levels are particularly closely monitored in baby foods and infant formulae. Phthalate concentrations in baby foods and infant formulae marketed in Denmark were investigated by PETERSEN and BREINDAHL (2000). The authors found at least one of the phthalates DBP, BBP or DEHP, in almost 50% of the samples of baby foods and infant formulae investigated, but their concentrations were relatively deep below TDI levels.

Phthalates in foods occur mainly as a result of contamination with phthalates from packaging materials. BALAFAS *et al.* (1999) measured phthalate concentrations in materials used as packaging for foods. The authors found total phthalate concentrations in packaging materials from 5 to 8160 $\mu\text{g/g}$. The phthalate most frequently found was DEHP. It was detected in all investigated samples at concentrations from 2 to 7058 $\mu\text{g/g}$. DBP and BBP are also commonly found in packaging materials. The authors stated that the highest phthalate concentrations were found in the food packaging materials made of printed polyethylene and they assumed that the main source of food contamination were the phthalates from the printing inks used.

It is very difficult to estimate the exposure of the Czech population to phthalates from foods

because very few data are available. On the basis of the analytical determinations of DEHP and DBP in several samples of meat, milk and meat and dairy products, ČERNÁ (2000) estimated the exposure of the Czech population to those phthalates at about 7.6% TDI.

Issues relating to the existence of phthalates in foods have received more and more attention and a number of measures to reduce the risk of contamination of foods with phthalates (e.g. restrictions on the use of tools and tubing of plasticised PVC and other plastic materials in the food industry, monitoring of phthalate concentrations in foods and beverages, etc.) have been adopted. It is very positive that phthalate concentrations in foods have decreased considerably in recent years as a result of those measures.

Legislation

Because phthalates are ubiquitous in the environment and the exposure to them is a potential health hazard, the industrial use of phthalates is being considerably regulated. A particularly sensitive approach is adopted with regard to the exposure of infants and children under three years of age. The most important document intended to reduce their exposure to phthalates is the decision of the EC Commission 1999/815/ES, which prohibited the placing on the market of toys and childcare articles intended to be placed in the mouth by children under three years of age made of soft PVC containing DINP, DEHP, DBP, BBP, di-*iso*-dodecyl phthalate (DIDP) or di-*n*-octyl phthalate (DNOP). The validity of that decision has been prolonged several times, most recently by decision 2004/781/ES. One of the reasons for the ban was the opinion of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) of 24th April 1998, in which CSTEE, among other things, set down TDI limits for the exposure of infants and children of a young age to some phthalates migrating from toys. The TDI limits set down by CSTEE were as follows (in µg/kg): 150 for DINP, 370 for DNOP, 50 for DEHP, 250 for DIDP, 850 for BBP and 100 for DBP. In the conclusion, CSTEE stated that even the low margins of safety (below 100) for DINP and DEHP give a reason for concern.

The opinion of CSTEE of 24th April 1998 also refers to TDI limits set down by the Scientific Committee for Food (SCF). DEHP is the only

phthalate for which the full TDI value in food (0.05 mg/kg body weight) has been set. TDI limits for other phthalates have been set as temporary values (TTDI) and it may be expected that they will be changed in accordance with new research findings. The TTDI values for DINP, DIDP, BBP and DBP are 0.03 mg/kg, 0.05 mg/kg, 0.1 mg/kg and 0.05 mg/kg, respectively. For the group of other phthalates used in packaging materials for food, the TTDI has been set at 0.05 mg/kg.

The issue of phthalate concentrations is dealt with also by Czech legislation. Maximum concentrations of phthalates in foods were originally set down by decrees 298/97 Sb. and 53/2002 Sb. of the Ministry of Health of the Czech Republic (1 mg/kg for spirits, and 2 or 4 mg/kg for foods). Although there is no mention of any limit values for phthalates in foods in the currently effective decree 305/2004 Sb. setting down the types of contaminating and toxicologically important substances and their concentration limits in foodstuffs, phthalates are dealt with in decree 38/2001 Sb on hygienic requirements for the products that come into contact with foods and foodstuffs. It follows from Appendix 3 to the decree that phthalates must not be used for the manufacture of plastic products intended to come into contact with foodstuffs. Appendix 11 to the same decree limits the use of phthalate plasticisers in the manufacture of varnishes. Areas of varnished surface of 1 dm² in size may contain a maximum of 25 mg phthalate plasticisers (di-alkylphthalates or dicyclohexylphthalate), and the overall migration limit for such plasticisers is 0.20 mg/dm².

The regulatory bodies in charge of the food safety in the Czech Republic are the State Agricultural and Food Inspection Authority and the State Veterinary Administration. In the past, phthalate concentrations in foods were monitored by the State Veterinary Inspection. Although phthalate concentrations in foods are not currently monitored on a systematic basis, the monitoring of phthalates in spirits and bottled water continues. This monitoring is organised by the State Agricultural and Food Inspection Authority.

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