# Phthalic Acid Esters (PAEs) in the Food Chain

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**Abstract**

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Phthalic acid esters (PAEs) rank among the primary risk pollutants and their adverse effects may endanger the environmental balance and affect the ontogenetic development of live organisms and their body functions. Therefore, the aim of this study was to monitor the occurrence of PAEs in packaging materials and plastics (infusion sets), to evaluate the accumulation and distribution of the most common phthalates such as DEHP (di-2-ethylhexyl phthalate) and DBP (di-*n*-butyl phthalate) in body tissues and organs of pigs and broiler chicks having been administered the phthalates *per os*, to assess the occurrence of PAEs in pig and cattle farms in the district of Hodonín (1997–1999), and to propose precautionary measures to mitigate the risk of PAE penetration into the food chain and the environments. DEHP and DBP contents in packaging materials ranged from 0.1 to 4259 mg DEHP, and from 0.1 to 1298 mg DBP per 1 kg printed packaging material, respectively. In haemodialysis patients, over 0.5 mg DEHP per 1 kg blood was found after three hours of haemodialysis. In combined feeds for farm animals (pigs, cattle, poultry), DEHP and DBP concentrations ranging from 0.07 to 1.77 and from 0.06 to 2.36 mg/kg feed, respectively, were detected. In all the food samples investigated, measurable levels of DEHP (less than 0.01–0.22 mg/kg sample) and DBP (less than 0.01 to 1.31 mg/kg sample) were found. In the experimental pigs and broilers, phthalates were distributed in all the organs monitored and the highest accumulation was found in adipose tissue as expected. All the samples withdrawn from farms in the Hodonín district had measurable phthalate concentrations; the hygienic limit (4 mg/kg) was exceeded in 2 samples of swine adipose tissue (4.26 and 6.92 mg/kg fresh sample) and in 1 sample of cattle adipose tissue (4.75 mg/kg fresh sample).

**Keywords**: phthalic acid esters; analytical methods; source of contamination; products and foods of animal origin

Phtalic acid esters (PAEs) belong to the most important contaminants of the environments and food chain in the developed industrial countries. Their toxicological effects and hygienic significance have been intensively investigated in recent years.

The industrial production of these substances began back in the mid 1930s. The production is simple and inexpensive. Most phthalates have excellent plastification and adhesion properties, therefore they are widely used in the chemical industry for the production of a wide assortment of products. These include plastic materials softened with phthalates, used in all the spheres of human activity (construction materials, components for various technologies, floor covering, furniture, leatherette, vessels, packaging, medical materials, etc.). Phthalates are utilised in the production of electrical cords, films, glues, paints, ink, var-

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nishes, coatings, adhesives, cosmetics, pesticides, repellents, dielectric media, impregnation means, elastomers, and so forth.

PAEs are a certain risk to the human body. Potential sources of PAEs include contaminated water, food, and air. Besides these, there are medical materials made from PVC softened with di-2-ethylhexyl phthalate (DEHP), such as infusion sets, peritoneal dialysis and haemodialysis sets, PVC bags for storing blood and blood derivatives, PVC tubes used as connections in parenteral nutrition and in many other medical operations. Even children's toys and many other products for children made from phthalate softened materials or dyed and printed with colours containing phthalates may be hazardous.

The maximum permissible content of phthalates in children's toys and childcare articles intended to be placed in the mouth by children under three years of age and made of soft PVC containing certain phthalates is 0.1 percentage by weight (2005/84/ES).

PAEs are biologically active compounds which dissolve in water to varying degrees depending on the configuration and length of ester chains (Howard *et al*. 1984). In the body, they are metabolised to toxic metabolites that react with biologically active substances and may impair vital functions of the body. The above-mentioned di-2-ethylhexyl phthalate (DEHP) and di-*n*-butyl phthalate (DBP) rank among the toxic and most frequently used phthalates. These substances are of lipophilic nature, and therefore they accumulate in adipose tissue. PAE residues have been detected in all the constituents of the environment, in live organisms, feeds, raw materials, and foods. An enormous production, blanket use, storage of PAE-containing waste in the environment, insufficient destruction by burning in waste combustion plants, and the possible negative efect of PAEs on human health pose great global environmental and health risks. The US Environmental Protection Agency (EPA) has listed six phthalic acid esters among the primary risk pollutants. These are dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), di-2-ethylhexyl phthalate (DEHP), di-*n*-octyl phthalate (DOP), and dibutylbenzyl phthalate (BBP).

An estimated world production of DEHP in 1994 was about 4 million tonnes (Klöpffer 1994). It is assumed that about 1.8 % of the yearly production leaks into the environment (Hubert *et al*. 1996).

Considering the occurrence and utilisation of phthalates, one may claim the people are exposed to their influence during medical interventions (release of DEHP form dialysis and transfusion sets) (Jarošová 1999), at work (manufacturing of plastic materials, rubber products, paints, etc.), and in everyday life due to the environmental contamination. The exposure of humans to the phthalates takes place via inhalation, and oral and skin absorption routes (Klimisch *et al*. 1992). Dermal and inhalative exposures are considered to be the major route of exposure to DEP that is found in hygienic products such as soap, shampoo, and conditioners. In contrast, with phthalates that are used mainly as plasticisers, such as DEHP, oral exposure predominates (Latini 2005). Because of their lipophilic nature, phthalates infiltrate mainly in foodstuffs containing fat (Sharman *et al*. 1994; Bluethgen 2000). Even the release of phthalates from packaging materials was demonstrated (Page & Lacroix 1992).

Acute toxicity of PAEs is low. The most commonly reported $LD_{50}$ for rats (Shibko & Blumenthal 1973) is 8–18 g/kg and 31 g/kg for DBP and DEHP, respectively. In 1991, the European Food Commission set Tolerable Daily Intake (TDI) levels by humans to 25 μg per kg body weight per day for DEHP and 50 μg per kg body weight per day for DBP.

Under a long-term exposure, the length and branching of the side chain cause an increase of undesirable side effects of PAEs on human body. In *in vivo* and *in vitro* experiments with live tissue cultures, the following undesirable effects were found due to a long lasting exposure to phthalates: teratogenicity and embryotoxicity, spermiotoxicity, hepatotoxicity, nephrotoxicity, and carcinogenity. Moreover, effects of PAEs on the cell membrane functions and other undesirable effects have been observed.

Several phthalates and their metabolic products have been shown to be developmental and reproductive toxicants affecting particularly male reproductive development and are suspected of having endocrine disrupting or modulating effects. The endocrine systems is responsible for the growth, sexual development and many other essential physiological functions both in males and females (Lovekamp-Swam & Davis 2003).

Usually, biological monitoring is used to confirm the exposure to foreign substances. Because phthalates are of lipophilic nature, their presence

is assumed in biological materials containing fat such as subcutaneous fat, maternal milk, and liver tissue.

## MATERIAL AND METHODS

### Material

***Packaging materials and plastics***. PAE contents were analysed in packaging materials for retail foods, collected in the sales network in Brno from June to August 1994. Foodstuffs were chosen that represent different commodities (confectionery, biscuits, meat and milk products, vegetables, potato crisps), are consumed mainly by children, and are packed in various types of printed packaging materials (plastic packages made from polypropylene, polyamide, polyvinylchloride, aluminium foil, and paper packages). Packages were submitted to relevant cleaning procedures prior to analyses (e.g. washed with water and dried, wiped with cotton dipped in ether). Samples of medical materials (haemodialysis, transfusion and infusion sets, connecting tubes) were obtained from the St. Anna Teaching Hospital in Brno.

***Combined feeds, raw materials of animal origin and foodstuffs***. Samples of combined feeds for farm animals were withdrawn from farms and feed mills in the region of South Moravia. Samples of animal origin (muscle and adipose tissue of swine, cattle and poultry) were collected at an abattoir located in the South Moravian region. Foodstuff samples were collected in the retail distribution network in Brno.

***Blood***. Blood, analysed for DEHP and MEHP, was withdrawn through haemodialysis sets from patients undergoing the haemodialysis therapy at the haemodialysis centre of the St. Anna Teaching Hospital in Brno. Blood withdrawn from pigs and broiler chicks included in the experiments carried out at the Veterinary Research Institute in Brno was also analysed for DEHP, MEHP and DBP.

***Experiment – pigs***. The administration of phthalates (DEHP and DBP) was monitored in two independent experiments. In each experiment, 6 piglets from the same litter of white thoroughbred race (3 males and 3 females) were used. Prior to the experiment, piglets were kept separately and later they were put in an experimental house at the Veterinary Research Institute in Brno. DEHP and DBP were dissolved in edible sunflower oil and added to the first portion of combined feeds in the morning and fed at an amount of 5 g per head, daily. Phthalate was orally administered to four experimental pigs for 14 days. Two control pigs received sunflower oil. Before starting the experiment, and then 7 and 14 days after the administration, the blood samples were analysed for DEHP, DBP, and MEHP. After 14 days of administration, two experimental and two control pigs were sacrificed. The remaining two experimental pigs were fed combined feeds, and sacrificed fourteen and twenty-eight days after the last day of phthalate administration. Blood and urine samples were collected before the slaughtering. After the slaughtering, the following samples were taken for PAE analysis: liver, kidney, lungs, brain, heart, muscle, adipose tissue. The samples of biological materials (blood and urine) were analysed right after the withdrawal.

***Experiment – broiler chicks***. ISA Vedette broilers were bought from Ing. J. Kocian's farm in Brno. Prior to the experiment, the broiler chicks were kept in the experimental house for a week so as to acclimatise. Later, they were divided into groups, 6 broilers in each. They all were kept in cages. DEHP and DBP, each at a dose of 100 mg, were separately added to the contents of gelatinous capsules filled with the BR2 combined feeds for broiler chicks. The gelatine shells, typically enclosing medicinal drugs, were used in the experiment to administer the above-mentioned, exactly weighed out quantity of a phthalate. Everyday, one capsule was given to each of the experimental broilers directly to the crop before morning feed. The control group broilers received capsules filled with feed only.

Each phthalate was orally administered to 18 broilers for 14 days. After this treatment, six broilers were sacrificed. Fourteen days after the last day of the phthalate administration, an other six broilers were killed. The remaining six broilers were slaughtered twenty-eight days after the last administration of a phthalate. Before slaughtering, animals were weighed and blood samples were collected by heart puncture for DEHP and DBP analysis. Livers were individually weighed and, due to their small weights pooled samples were analysed (six livers from each group). Muscle (pooled samples of breast and thigh muscles), skin (thoracic area), and mesenterial fat were examined for DEHP and DBP. In broilers, which were killed immediately after the phthalate application, tissues (besides liver) and blood were used for the determination of DEHP and DBP.

Samples of biological material (blood) were analysed right after the withdrawal.

***Swine and cattle farms.*** Between 1997 and 1999, in three swine farms (Dubňany – D, Milotice – M, Terezín – T) and two cattle farms (Násedlovice – Na, Nesyt – Ne), situated in the Hodonín district, 64 environmental samples were analysed for the phthalate contents. Of these, 29 were samples of pig and cattle combined feeds, 18 samples of fresh cow milk, 12 samples of porcine and bovine adipose tissue, and 5 samples of plastics. Feed samples were withdrawn directly from feed troughs or gutters, milk was withdrawn from the bulk tank, samples of fat were withdrawn at the abattoir (subcutaneous fat in pigs, renal fat in cattle). Samples of plastic barn equipment were also collected (slats, troughs, bars).

### Methods

Analytical methods were developed, validated and introduced to determine PAE contents. Detailed descriptions of the analytical procedures are included in the studies published previously (Gajdůšková *et al.* 1996; Jarošová *et al.* 1998, 1999).

Tissue and food samples were collected immediately after the slaughtering. Then, they were homogenised, put in Petri dishes and left to freeze. Frozen samples and feed samples were lyophilised and PAE residues were subsequently extracted by *n*-hexane. PAE was separated from its co-extracts by means of gel permeation chromatography into gel Bio beads S-X3. To complete the cleaning of feed eluates, concentrated sulphuric acid was used.

Blood samples were analysed immediately after the collection. For a quick determination of blood DEHP, DBP, and MEHP, a procedure based on the extraction of PAEs by ethyl acetate was used. Organic phases were transfered to heart-shaped flasks with ground joints and concentrated in a rotary vacuum evaporator and evaporated until dry by a nitrogen pressure stream. The residue after evaporation was dissolved in a suitable amount of acetonitrile for HPLC analysis. MEHP was extracted after DEHP and DBP by adding citrate buffer.

Urine samples were examined immediately after the collection. An amount of 250–500 ml of urine was diluted with the same quantity of distilled water. DEHP and DBP were extracted with 30, 20, and 20 ml of dichlormethane in a funnel. All the extracts were then concentrated in a heart-shaped flask in a rotary vacuum evaporator (water-bath temperature 40°C), and evaporated until dry under the pressure of a nitrogen stream. The residue after evaporation was dissolved in 100–500 µl of acetonitrile for HPLC determination. After DEHP and DBP extraction, phosphoric acid was added to the urine solution to adjust it to pH 2. Then, MEHP was extracted by dichlormethane using the previously mentioned procedure. PAE determination by HPLC method was in accordance with PAE determination in blood.

PAEs in all the samples were determined by high-performance liquid chromatography with Separon SGX C 18 and UV detection at 224 nm (diode array detector). For the assessment of the results, the program STAT Plus, version 1.01 (Matoušková *et al.* 1992) was used.

## RESULTS AND DISCUSSION

### Packaging materials and plastics

Total of 42 samples of packaging from confectionery, biscuits, meat, and milk products, frozen products, vegetables, potato crisps, and other snacks eaten mainly by children were analysed.

PAE contents were monitored separately in printed and blank parts of packaging. In general, higher DEHP concentrations were found in packaging materials tested as compared with DBP concentrations. Printed packaging materials where colours with added phthalates were used to ensure good adhesion properties, showed higher PAE concentrations than the non-printed ones (Table 1).

Variable occurrence and concentrations of DEHP and DBP were demonstrated in food packaging materials that are in a direct contact with food (Gajdůšková *et al.* 1996; Jarošová *et al.* 1996). PAE contents found in the whole packaging do not necessarily indicate that the packaging is not suitable for foods. Some packagings include barrier layers that prevent or decrease the PAE migration.

Table 1. Contents (in mg/kg) of DEHP and DBP in packaging materials

| Sample | DEHP | DBP |
|---|---|---|
| Packing with printing | 0.1–4259 | 0.1–1298 |
| Packing non-printed | 0.1–1881 | 0.1–686 |

In co-operation with human medicine, the issue of the phthalate presence in medical materials and a possibility of the contamination of the body with phthalates from those have been studied. Most medical sets include products made from PVC (tubes, connections, bags). 60 haemodialysis and infusion sets were examined, coming from the companies Gambro (Sweden), Bieffe Medita (Italy), Braun Melsungen (Germany), Baxter (USA) and Fresenius (Germany). In some cases, DEHP contents were over 40% (on weight basis) (Jarošová 1999).

### Combined feeds, raw materials of animal origin and foodstuffs

In combined feeds for farm animals (pigs, cattle, poultry), DEHP concentrations from 0.07 to 1.77 mg/kg feed and DBP concentrations from 0.06–2.36 mg/kg feed were found. DEHP and DBP levels found in compound feed confirmed a necessity to check the phthalate residue levels in feeds (Raszyk *et al*. 1998; Jarošová *et al*. 1998).

By analysing muscle and adipose tissues of animals from South Moravian farms, measurable concentrations of DEHP and DBP in all the samples analysed were determined (Table 2). High concentrations of DEHP and DBP in muscle and adipose tissue of farm animals correlate with their relatively high concentrations in feeds. Farm animals may also be contaminated from other sources in the farm environment (Raszyk *et al*. 1998; Jarošová *et al*. 1998).

In addition to raw materials of animal origin, foods packed in different types of packaging and collected from the food distribution network or delivered directly from the food-processing plants were analysed (Jarošová *et al*. 1997). In all the food samples, measurable concentrations of DEHP (less than 0.01 to 0.22 mg/kg sample) and DBP (less then 0.01 to 1.31 mg/kg sample) were found. The results were included in the "System of Control of Foreign Substances in the Food Chain". They were put in the databasis of the Czech State Veterinary Administration, evaluated and listed in the Bulletin of Czech State Veterinary Administration together with the results from the State Veterinary Institutes that took part in the monitoring of phthalates in food.

Table 2. Concentrations of DEHP and DBP (mg/kg original sample) in muscle and adipose tissue of animals

| Sample | DEHP | DBP |
|---|---|---|
| Bovine fat | 0.55–1.52 | 1.76–4.17 |
| Porcine fat | 0.20–0.80 | 1.37–6.12 |
| Poultry fat | 0.20–1.71 | 0.20–0.68 |
| Poultry muscle | 0.02–0.30 | 0.08–0.24 |

### Blood

During haemodialysis procedures, DEHP is extracted from PVC materials by the lipid components of blood. It was demonstrated that after three hours of haemodialysis, up to over 0.5 mg DEHP were contained in 1 kg blood. DEHP levels in blood of the patients depended on the provenance of a haemodialysis set used. The level of extraction of DEHP from PVC probably depends on the components and technologies used to manufacture the PVC sets (Jarošová 1999).

In blood stored in PVC bags and intended for transfusion, 7.8 mg DEHP/kg whole blood were found. The results are alarming because human health may be endangered. PVC materials, or other plastics containing phthalates, should be replaced promptly with materials that do not contain hazardous toxic substances (Jarošová 1999).

### Experiments – pigs

During the experiment, animals showed no clinical signs of disease. The distribution of DEHP and DBP in the tissues, in the original sample, the phthalate levels in the experimental animals on day 14 of administration, and 14 and 28 days after the last day of administration are given in Table 3 and in publications of Gajdůšková *et al*. (1996) and Jarošová *et al*. (1998, 1999). During the 14 days of the phthalate administration, DEHP was confirmed in the blood and urine by the presence of MEHP. The levels of blood and urine DEHP and DBP in the samples were mostly under the detection threshold of the methods used.

The highest DEHP level was in the subcutaneous and renal fat, muscle, heart and lungs. DEHP amount in liver and kidneys was low, probably due to MEHP formation. Fourteen and twenty-eight days after the last administration, DEHP concentration in the organs decreased significantly. In muscle and adipose tissue, the DEHP value decreased by about 50%. The highest DBP amount was found in the muscle and subcutaneous tissue, whereas in the

Table 3. Concentrations of DEHP and DBP (mg/kg original sample) in the tissues of experimental pigs after 14 days of application (5 g per head, daily) and 14 and 28 days after the last application

| Sample | DEHP | | | DBP | | |
|---|---|---|---|---|---|---|
| | after 14 days of application | 14 days after the last application | 28 days after the last application | after 14 days of application | 14 days after the last application | 28 days after the last application |
| Muscle | 1.33 | 0.48 | 0.46 | 1.64 | 0.04 | 0.46 |
| Renal fat | 19.10 | 6.41 | 6.90 | 0.15 | 0.30 | 0.77 |
| Subcutaneous fat | 13.29 | 5.63 | 6.31 | 9.53 | 2.04 | 2.01 |
| Kidneys | 0.22 | 0.08 | <0.01 | 0.05 | 0.04 | 0.02 |
| Lungs | 1.02 | 0.20 | 0.20 | 0.06 | 0.03 | 0.02 |
| Brain | 0.03 | < 0.01 | < 0.01 | 0.24 | 0.48 | 0.12 |
| Heart | 0.36 | 0.31 | < 0.01 | 0.10 | 0.32 | 0.38 |
| Liver | 0.10 | < 0.01 | < 0.01 | 0.05 | 0.11 | 0.04 |

renal fat, it was significantly smaller as compared to DEHP. We have no explanation for this. DBP, in comparison with DEHP, is uniformly distributed in the organs and tissues. Thus, we can assume that it shows a higher persistence in the organs and tissues than DEHP. Concerning the rate of DBP metabolism, its half-time elimination and specific metabolites (for example the monobutylester), no pharmacokinetic studies were available. The confirmation of the lipophilic character of DEHP and DBP and their presence and distribution in tissues and organs in the dependence on the fat amount and DEHP or DBP persistence (values determined after 14 and 28 days from the application period in tissues and some organs) can be considered as original results about phthalate accumulation in the body of pigs. Accordingly, the presence of toxic phthalates in the adipose tissue and muscle of pigs can be considered as an alarming situation from the point of view of food hygiene.

**Experiment – broiler chicks**

There was a high cumulation of DEHP in the mesenterial fat, skin and muscle (Table 4) after the administration. The DEHP level in liver was low. Fourteen and twenty-eight days after the last application of DEHP, the amount of DEHP decreased in all the tissues examined (Table 4). This is attributed to the formation of MEHP and the so called "diluting factor" due to the increased weights of broilers. Although an equal dosage of DBP was applied orally, the distribution of DBP in liver and tissues was significantly different from that of DEHP (Table 4). DBP cumulation was eight times lower than that of DEHP. DBP was equally

Table 4. Concentration of DEHP and DBP (mg/kg original sample) in the tissues of experimental broiler chicks after 14 days of application (100 mg per head, daily) and 14 and 28 days after the last application

| Sample | DEHP | | | DBP | | |
|---|---|---|---|---|---|---|
| | after 14 days of application | 14 days after the last application | 28 days after the last application | after 14 days of application | 14 days after the last application | 28 days after the last application |
| Muscle | 1.93 | 0.42 | 0.22 | 0.19 | 0.11 | 0.08 |
| Skin | 8.28 | 3.2 | 2.75 | 0.90 | 0.63 | 0.62 |
| Mesenterial fat | 18.2 | 10.65 | 5.95 | 3.13 | 1.54 | 0.82 |
| Liver | 0.32 | 0.25 | 0.04 | 0.27 | 0.09 | 0.07 |

distributed in adipose tissues, muscle and liver. Fourteen and twenty-eight days after the last administration, there was a high DBP level corresponding to the dilution ratio by reason of the increased weights of broilers. The detected values in the muscle and skin indicate the persistence of DBP and its slow metabolism. Neither DEHP nor DBP was detected in the blood of experimental and control broilers. The mean concentrations of DEHP and DBP ($n$ = 6) in the tissues are given in Table 4. The variability of the concentration of DEHP (muscle: 0.84–3.30, skin: 3.41–14.34, mesenterial fat: 8.80–28.73) and of DBP (muscle: 0.12–0.35, skin: 0.64–1.41, mesenterial fat: 2.56–3.95) in the tissue of the broilers in one group, can be probably attributed to various individual enzyme activities and other body functions which are involved in the metabolism of xenobiotics and excretion from the body. During their study, Barron *et al*. (1995) confirmed great differences in DEHP levels in the tissues of fish.

In the body tissues of the control pigs and broilers, measurable concentrations of DEHP and DBP were detected by organ and tissue analyses and the accumulation of phthalates from contaminated feed was confirmed. All the samples of combined feeds, given to the treated and control animals, contained DEHP and DBP (Table 5). The confirmation of the accumulation and distribution of toxic phthalates in the body of farm animals after an oral administration is significant in terms of the health safety of raw materials and foodstuffs of animal origin.

Table 5. Contents of DEHP and DBP (mg/kg feed) in commercial feed given to the treated and control animals (ČOS, A1, A2, A3 commercial feeds for pigs; BR2 comercial feed for broilers)

| Sample | DEHP | DBP |
|---|---|---|
| 1 – ČOS | 0.49 | 0.45 |
| 2 – A1 | 0.24 | 0.47 |
| 3 – A2 | 0.35 | 0.61 |
| 4 – A3 | 0.44 | 0.66 |
| 5 – A3 | 0.34 | 0.32 |
| 6 – A3 | 0.45 | 0.52 |
| 7 – A3 | 0.33 | 0.96 |
| 8 – BR2 | 1.77 | 2.36 |
| 9 – BR2 | 0.24 | 0.06 |

**Evaluation of PAE occurrence in swine and cattle farms in the district of Hodonín**

From 1997 to 1999, in the Hodonín district, average concentrations of DEHP and DBP in combined feeds for swine ($n$ = 21) were 0.165 mg/kg and 0.126 mg/kg, respectively, and in combined feeds for cattle ($n$ = 8) 0.151 mg/kg and 0.379 mg/kg, respectively (Table 6).

Measurable concentrations of DEHP and DBP were detected in all the analysed samples of adipose tissue of pigs and cattle from the farms in the Hodonín district. In swine subcutaneous fat ($n$ = 6), the average contents of DEHP and DBP were 0.510 and 3.360 mg/kg, respectively. In bovine renal fat ($n$ = 6), 0.790 mg/kg DBP and 2.540 mg per kg, respectively were found (Table 6) (Ulrich *et al.* 1999).

The samples of plastics withdrawn in the barns contained 26.040 mg/kg DEHP and 2.560 mg/kg DBP (Table 6). PAEs are not chemically bound in plastics and can be leached out with water. Their weight proportions in plastics ranged from 0 to 40% (Pearson & Trissel 1993).

So far, in the Czech Republic the hygienic limits for phthalate contents in farm animal feedstuffs

Table 6. Contents of DEHP and DBP (mg/kg) in combined feeds, fat tissues of swine and cattle, raw bulk milk, and plastics in the district of Hodonín in 1997–1999

| Sample | $n$ | DEHP | DBP | ∑ DEHP + DBP |
|---|---|---|---|---|
| Combined feeds for swine | 21 | 0.165 | 0.126 | 0.291 |
| Combined feeds for cattle | 8 | 0.151 | 0.379 | 0.530 |
| Raw bulk milk | 18 | 0.386 | 0.299 | 0.685 |
| Porcine fat | 6 | 0.510 | 3.360 | 3.870 |
| Bovine fat | 6 | 0.790 | 2.540 | 3.330 |
| Plastics | 5 | 26.480 | 2.560 | 29.040 |

have not been set. Over the period of this study, a hygienic limit, expressed as total phthalate content (DEHP, DBP), was 2 mg/kg fresh sample for milk, milk products, meat, meat products, and 4 mg/kg fresh sample for fats, liver and kidneys (Annex No. 3 to the Decree of the Ministry of Agriculture of the Czech Republic No. 298/1997 Coll., implementing the Food Law – Act No. 110/1997 Coll.).

Hygienic limits were exceeded in 2 samples of porcine fat (4.26 and 6.92 mg/kg) and 1 sample of bovine fat (4.75 mg/kg fresh sample). Raw bulk milk samples showed 34% of the hygiene limit, bovine fat samples 83%, and porcine fat samples 97%.

In 1996 and 1997 in selected State Veterinary Institutes in the Czech Republic, 5 samples of fresh meat, 33 samples of meat products, 29 samples of poultry meat and organs, 28 samples of milk, and 22 samples of milk products were examined (Drápal & Valcl 1998). Average levels of total phthalates (DEHP, DBP) in the above-mentioned samples were as follows: in meat 1.280 mg/kg fresh sample, in meat products 1.071 mg/kg fresh sample, in poultry meat and offal 0.666 mg/kg fresh sample, in milk 0.272 mg/kg fresh sample, and in milk products 0.311 mg/kg fresh sample.

## CONCLUSION

In order to prevent the occurrence of PAEs, and on the basis of the comprehensive knowledge of the effects phthalates exert on living organisms as well as of the results of the studies of the occurrence of phthalates in the environment and food chain, it is necessary to monitor DEHP and DBP levels in food and feed ingredients to identify hazardous substances and sources of phthalate contamination. A suitable indicator of DEHP and DBP contamination is adipose tissue (subcutaneous fat in pigs, renal fat in cattle, skin in poultry) and muscle. To reduce the PAE contamination risk, it is necessary to monitor packaging materials used for storing feedstuffs, raw materials and foodstuffs, and to watch closely coloured prints, adhesives and other elements that are in contact with feeds and foods.

The experiments were carried out at the Veterinary Research Institute in Brno.

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