

## The distribution and accumulation of phthalates in the organs and tissues of chicks after the administration of feedstuffs with different phthalate concentrations

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**ABSTRACT:** For the monitoring of distribution and accumulation of phthalic acid esters (PAE) in animal tissues, samples of muscle, mesenteric fat (fat), skin and liver from broiler chicks ROSS 308 were used. The chicks were divided into four groups (50 chicks each). All the chicks were given commercial diets (complete feed, KKS) for broiler chicks (starter – BR1; grower – BR2 and finisher – BR3). The experimental diets were supplemented with vegetable oil (RV) with low (group N) or high (group V) phthalate contents, or animal fat with a high phthalate content (group Z). Neither the control diets (K), nor the grower (BR1) diets contained vegetable oils or animal fat. The N chicks were given the grower (BR2) and finisher (BR3) diets supplemented with 5% and 3% vegetable oil, respectively. The V chicks were given BR2 and BR3 diets with 5% and 3% vegetable oil, respectively. The Z chicks were given BR2 and BR3 diets with 5% and 3% animal fat, respectively. The chicks were fattened till 42 days of age. Dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) were found in the tissues of chicks in all the experimental groups. The DBP content in the muscle ranged from 0.03 to 0.55 mg/kg, in the adipose tissue from < 0.20 to 2.56 mg/kg, in the skin from < 0.20 to 1.49 mg/kg, and in the liver from 0.03 to 0.13 mg/kg. The content of DEHP in the muscle ranged from 0.03 to 1.15 mg/kg, in the adipose tissue from 0.25 to 9.85 mg/kg, in the skin from < 0.20 to 4.68 mg/kg, and in the liver from 0.16 to 0.24 mg/kg. The highest concentrations of DBP of  $1.28 \pm 1.00$  mg/kg of fresh sample (an average value from eight chicks) was determined in the adipose tissue of V chicks. The highest concentration of DEHP of  $3.27 \pm 2.87$  mg/kg of fresh sample (mean of eight chicks) was also determined in the V group. The accumulation of DEHP was 3.2; 2.6 and 2.9 times higher than that of DBP in the muscle, adipose tissue and skin, respectively. The V and Z chicks showed higher phthalate contents (the sum of DBP and DEHP) in the adipose tissue, skin and liver than the K and N chicks.

**Keywords:** DBP; DEHP; analysis; monitoring; contamination; HACCP; feed material

Phthalates are now widely used all over the world, not only as plasticizers but also as additives in industrial products, including food and personal care products (Petersen and Breindahl, 2000). Phthalic acid esters are found in huge range consumer products including floor- and wall-covering, furnishing, toys, car interior, clothing etc. (Afshari et al., 2004; Schettler, 2006).

Phthalates are also applied to paints and lacquers, adhesives, printing inks etc. As the phthalates are

not chemically bound in the polymers, migration or emission of the phthalates from the product to the environment is likely to occur. Although phthalates are non-persistent chemicals that are rapidly metabolised, contamination of the environment is significant due to the widespread use. Among the most important phthalates are dibutyl phthalate and di-2-ethylhexyl phthalate. In Europe, most of the food in contact with plastic contain DEHP and DBP. These are also found in common food prod-

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ucts, such as cereals, bread, biscuits, cakes, nuts, spices, fat and oil in amounts up to about 10 mg/kg (Wormuth et al., 2006). In animal additives, effects of phthalates have been demonstrated (Borch et al., 2004). Humans are exposed to phthalates from a wide range of consumer products. It is assumed that phthalates and other endocrine disrupting chemicals might have contributed to adverse trends in reproductive medicine, which show an increase in testicular cancer and hypospadias as well as a decrease in sperm counts (Sharpe and Irvine, 2004; Lottrup et al., 2006). Epidemiologic studies in children (Jaakkola and Knight, 2008) show associations between indicators of phthalate exposure at home and risk of asthma and allergies (Takano et al., 2006). Decomposing pattern depends on the length of side chain. DBP and DEHP are teratogenic, mutagenic and carcinogenic (Yin et al., 2003). The main metabolites of long chain phthalates are oxidized metabolites (Wittassek and Angerer, 2008). Short branched diesters are mainly excreted in urine as its monoester phthalates (Frederiksen et al., 2007). Recent studies have shown that infants are exposed to phthalates and their metabolites through breast milk, infant formulae and baby food (Schettler, 2006). Becker et al. (2004) found that the oxidative metabolism of DEHP is dependent on age. In children the ratios of oxidized DEHP metabolites to MEHP generally increased with decreasing age. Age-dependent metabolism of phthalates may also have relevance to health: the oxidation products are longer in the human body than the simple monoesters, they might be more toxic (Stroheker et al., 2005).

The data on the occurrence of DBP and DEHP in commercial complete feeds for swine, cattle and poultry were given in our previous publications (Raszyk et al., 1998; Jarosova, 2006).

The aim of this study was to investigate the distribution and level of accumulation of DEHP and DBP in tissues of chicken broilers depending on dietary phthalate contents in the fattening period.

## MATERIAL AND METHODS

### Chemicals

For the analytical determination of PAE the analytical standards on purity of over 99.9%, DEHP and DBP from Supelco (USA) were used. Basic and working solutions were diluted with acetonitrile and stored in the refrigerator. Acetone, dichloromethan,

*n*-hexane of purity for residues and acetonitrile of gradient purity LC.

### Experiment – chicks

All the experimental chicks were fed with commercial diets for broiler chicks (BR1; BR2 and BR3), according to the age. The experimental diets (KKS) were supplemented with vegetable oil (RV) with low (group N), or high (group V) phthalate contents, or animal fat with a high phthalate content (group Z). The vegetable oils added were received from registered manufacturers of feed materials. RV rape seed oil that was intended both for human nutrition and animal nutrition was transferred right after the pressing into two tanks and stored there. One tank was made of steel (a low phthalate content), the other one was made of plastic (a high phthalate content). Vegetable oils were collected during the expedition from the tanks after ca. one month of storage.

The trial included 200 one day old chicks ROSS 308. The chicks were divided into four groups (50 chicks each):

#### Group K

from Day 1 to Day 21 the chicks received BR1 diet (without fat supplementation)

Days 22–35: BR2 diet (without fat supplementation)

Days 36–42: BR3 diet (without fat supplementation)

#### Group N

Days 1–21: BR1 (without fat supplementation)

Days 22–35: BR2 (with 5% low phthalate vegetable oil)

Days 36–42: BR3 (with 3% low phthalate vegetable oil)

#### Group V

Days 1–21: BR1 (no fat supplementation)

Days 22–35: BR2 (with 5% high phthalate vegetable oil)

Days 36–42: BR3 (with 3% high phthalate vegetable oil)

#### Group Z

Days 1–21: BR1 (no fat supplementation)

Days 22–35: BR2 (with 5% high phthalate animal fat)

Days 36–42: BR3 (with 3% high phthalate animal fat)

The contents of DBP and DEHP (mg/kg) in the feeds given to Control chicks (K), low phthalate

chicks (N) and high phthalate chicks (V) and chicks receiving animal fat (Z) are listed in Table 1.

The ROSS 308 chicks were kept in the authorized experimental hall at the University of Veterinary and Pharmaceutical Sciences Brno (VFU Brno) on deep litter; the groups were separated, each group had a separated feeding and drinking system (tube feeders and bell drinkers). Feed and water were provided *ad libitum*. The chicks were fed with commercial diets (complete feed) and had a free access to drinking water. Temperature and relative humidity were continuously monitored and recorded by the device Testo Loger 175-H2, and corresponded with requirements defined in the Ross 308 management guide. The controlled lighting regimen was used – 23 hours light, one hour darkness. The air exchange in the hall was provided by a continuously working ventilation system.

During the trial the health of chicks was monitored daily and no clinical signs of disease were observed.

For the chemical analysis, eight chicks (four females and four males) were selected randomly from each group. Mean final live weights of control and fat treated chicks are listed in Table 2, and were ranging from 1 560 g to 3 140 g. After the stunning, the chicks were scalded with hot water and plucked carefully to avoid the skin damage. Muscle (pooled sample of breast and thigh muscle from the left

carcass half), skin and mesenteric fat were analysed for the contents of DEHP and DBP. Livers, because of low weight, were analysed as pooled samples (the homogenate of eight livers from each group).

In the control (K) and fat treated (N, V, K) groups, the determination of DEHP and DBP was performed individually for every chick.

### The methods used

The tissue samples were collected right after the slaughter, homogenized, weighed, placed in aluminium dishes (50–300 g) and frozen. Gradually, the frozen samples were freeze-dried and subsequently PAE residues were extracted with *n*-hexane. The feed samples were also freeze-dried before the extraction with *n*-hexane. PAE were separated from co-extracts by gel permeation chromatography with the gel Bio Beads S-X3. For the final purification of the eluates, the purification procedure with concentrated sulphuric acid was used. For the determination of PAE, the high performance liquid chromatography (HPLC) was used, with the liquid chromatographer Agilent Technologies LC/MSD VL, Cogent e-Coolum C 18, graining 5  $\mu$ m, length 150 mm, Super Link with UV and MS detection, mobile phase acetonitrile: water (99 : 1). The assessment was done with the Agilent chemstation software.

Table 1. Concentrations of DBP and DEHP (mg/kg) in fats and complete feeds, ingested by the chicks of groups K, N, V and Z from Day 1 to Day 42

Feed	DBP	DEHP	$\Sigma$ DBP + DEHP
	(mg/kg)		
BR1 – K, N, V, Z	0.96	0.48	1.14
BR2 – K	1.37	0.52	1.89
BR3 – K	1.02	0.76	1.78
Vegetable oil with low phthalate content – N	15.56	2.25	17.81
Vegetable oil with high phthalate content – V	51.35	7.0	58.35
Animal fat with high phthalate content – Z	43.28	2.10	45.38
BR2 – N	2.15	0.63	2.78
BR3 – N	1.49	0.83	2.32
BR2 – V	3.94	0.87	4.81
BR3 – V	2.56	0.97	3.53
BR2 – Z	3.53	0.63	4.16
BR3 – Z	2.32	0.82	3.14

Table 2. Mean live weight (kg) and standard deviation in control chicks (K) and tested chicks (N, V, Z),  $n = 8$ 

	K	N	V	Z
Mean live weight (kg)	2.42	2.39	2.30	2.50
S.D.	0.33	0.30	0.43	0.35

For the determination of PAE, routine methods for determination of phthalates in foodstuffs were used (Jarosova et al., 1998, 1999).

Concurrently, for each sample dry matter and fat (Soxhlet) were determined.

All the samples were double-analysed. DEHP and DBP concentrations are related to the fresh sample.

The data obtained were statistically evaluated by the software Unistat 5.1. For the data processing ANOVA was used and subsequently multiple comparison by means of Tukey-HSD to find pairs of groups with statistically significant differences (Zar, 1999).

## RESULTS

Concentrations of DBP and DEHP in muscle, fat, skin and liver of control chicks (K), chicks fed with low phthalate diet (N), chicks fed with high phthalate diet (V) and chicks fed with animal fat supplemented diet (Z) are given in Table 3.

Table 3. Mean values of DBP and DEHP ( $\bar{x} \pm \text{S.D.}$ ) in mg/kg of fresh sample in muscle, fat, skin and liver of control chicks (K), low phthalate diet fed chicks (N), high phthalate diet fed chicks (V), chicks fed the diet supplemented with animal fat (Z),  $n = 8$ 

Samples	K	N	V	Z
	control	low phthalate diet	high phthalate diet	animal fat
	(mg/kg)			
Muscle				
DBP	0.22 ± 0.10	0.08 ± 0.04	0.15 ± 0.07	0.22 ± 0.19
	(0.10–0.36)	(0.03–0.15) <sup>a,b</sup>	(0.07–0.33)	(0.07–0.55)
DEHP	0.35 ± 0.08	0.08 ± 0.04	0.32 ± 0.18	0.39 ± 0.30
	(0.20–0.45)	(0.03–0.14)	(0.09–0.62)	(0.19–1.15)
Fat				
DBP	0.55 ± 0.36	0.59 ± 0.46	1.28 ± 1.00	0.89 ± 0.74
	0.24–1.45	< 0.20–1.70	0.28–2.56	0.34–2.54
DEHP	1.38 ± 0.91	1.92 ± 1.35	3.27 ± 2.87	1.85 ± 1.27
	0.31–3.08	0.67–4.96	0.71–9.85	0.25–3.84
Skin				
DBP	0.39 ± 0.23	0.51 ± 0.39	0.57 ± 0.37	0.44 ± 0.17
	< 0.20–0.78	0.20–1.49	0.23–1.14	0.21–0.73
DEHP	1.18 ± 1.36	1.10 ± 0.55	1.38 ± 1.07	1.60 ± 1.01
	0.31–4.68	0.58–1.95	0.33–3.61	< 0.20–3.02
Liver*				
DBP	0.05	0.03	0.11	0.13
DEHP	0.16	0.16	0.24	0.23

Limit of determination of BP and DEHP in fat matrices: 0.2 mg/kg

Limit of determination of DBP and DEHP for animal and vegetable materials with low fat content: 0.03 mg/kg

<sup>a</sup>statistically significant as related to K ( $P < 0.05$ )

<sup>b</sup>statistically significant as related to Z ( $P < 0.05$ )

\*analysed as pooled sample

The highest accumulation of DBP was found in the chicken fat. Mean DBP concentrations in the fat in different groups ranged from 0.55 to 1.28 mg/kg. The highest DBP concentration in the fat was found in V chicks ( $1.28 \pm 1.00$  mg/kg). The second highest DBP concentration was found in the skin. Mean DBP concentrations in the skin of chicks in different groups were within a narrow range of 0.39 to 0.57 mg/kg. The highest DBP concentration in the skin was found in the group V, too ( $0.57 \pm 0.37$  mg/kg). Mean DBP concentrations in the muscle of chicks in different groups were ranging from 0.08 to 0.22 mg/kg. The lowest DBP concentrations were found in the chicken liver. Mean DBP concentrations in the liver of chicks of different groups were ranging from 0.03 to 0.13 mg/kg.

DEHP, similarly to DBP, was the most accumulated in the fat. Mean concentrations of DEHP in the fat of different groups were ranging from 1.38 to 3.27 mg/kg. The highest concentration of DEHP in fat was found in the group V, namely  $3.27 \pm 2.87$  mg/kg. The second highest DEHP concentration was found in the skin. Mean concentrations of DEHP in the skin of chicks in different groups were ranging from 1.10 to 1.60 mg/kg. The highest DEHP concentration in the skin was found in the group Z, namely  $1.60 \pm 1.01$  mg/kg.

Mean concentrations of DEHP in the muscle of chicks of different groups were ranging from 0.08 to 0.39 mg/kg. The highest DEHP concentrations in the muscle were found in the Z chicks, namely  $0.39 \pm 0.30$  mg/kg. The lowest DEHP concentrations were found in the liver. Mean concentrations of DEHP in the liver of chicks of different groups were ranging from 0.16 to 0.24 mg/kg.

In the muscle, fat, skin and liver, the content of DEHP was always higher than that of DBP, namely 1.0 to 2.1 times, 2.1 to 3.2 times, 2.2 to 3.6 times and 1.8 to 5.3 times, respectively.

The highest accumulation of phthalates (a sum of DBP and DEHP) was found in fat and skin across the groups. The chicks that were fed with high phthalate content diets (V), as well as those fed the diets supplemented with animal fat (Z) showed markedly higher phthalate contents in fat, skin and liver than the control chicks (K) and chicks fed low phthalate diets (N).

The differences in DBP and DEHP found were not significant. An exception was DEHP concentration in muscle samples from the group N that showed significantly lower values than the groups K and Z; the concentrations of DBP and DEHP in muscle were approaching the limit of determination.

## DISCUSSION

The initial impulse to perform this experiment with chicks was the finding that raw materials such as vegetable oils, animal fats, wheat and corn are significantly contaminated with phthalates. For this reason the control chicks (K) were given commercial feedstuffs with no added fat. The tested chicks (groups N, V, Z) were given commercial diets supplemented with industrial raw materials, i.e. rape seed oil and animal fat. These fat sources contained measurable levels of a sum of DBP and DEHP (Table 1) that exceeded 1 mg/kg feed.

After the pressing, the rape seed oil was stored either in a metal tank (for the N chicks), or in a plastic tank (for the V chicks). The main source of contamination of vegetable oil was a plastic tank where the oil was stored before the delivery (Harazim et al., 2008).

We found that some raw materials used for the compound feed manufacture contained high phthalate levels (a sum of DBP and DEHP): wheat 4.06 mg/kg, corn 4.37 mg/kg. Because cereals make up to 70% commercial compound feed, they may pose a significant risk not only to farm animals, but also to the human food chain.

Feedstuffs are regarded as the main source of phthalates for fattening farm animals (Raszyk et al., 1998). Beside contaminated feeds there can be other secondary sources that can supply high levels of phthalates directly in the stable environment on farms (plastics softened with up to 40% DEHP, paints, lacquers).

In 1997 and 1998, samples of plastic materials from the interior equipment of stables (slats, troughs, bars) were collected on swine and cattle farms. Mean DBP concentration in plastics was 2.56 mg/kg of original sample, whereas mean DEHP concentration was markedly higher – 26.48 mg/kg of original sample.

In the previous study (Jarosova et al., 1999), the distribution and accumulation of DEHP and DBP in pig and broiler tissues after oral administration of phthalates was monitored. The contents of DBP in the skin, muscle, mesenteric fat and liver were 0.9, 0.19, 3.13 and 0.27 mg/kg of fresh sample, respectively. In this study, the phthalate levels found were slightly lower, mainly those of DEHP and DBP in the skin and fat, which was related to the contents of PAE in the diet.

In the complete feeds, vegetable oil and animal fat that were fed to the chicks, the contents of DBP



were always higher than those of DEHP. On the contrary, in the muscle, fat, skin and liver of the chicks in all the groups (K, N, V, Z), the content of DEHP was always higher than that of DBP. DBP is generally regarded as a more frequently occurring environmental contaminant.

The differences in the accumulation of DEHP and DBP in the body of broilers can be caused by individual differences in metabolism, the ratio between the accumulation and elimination from the body, as well as by differences in physical and chemical properties between the phthalates. DBP has a smaller molecule with a shorter, non-branched chain, which enables partial water solubility. DEHP is water insoluble.

The values of DEHP in the liver in all the groups of chicks ranged between 0.16 and 0.24 mg/kg of fresh sample. They were substantially lower than those in other tissues, probably due to metabolic breakage of DEHP to mono-2-ethylhexyl phthalate (MEHP). MEHP was detected in the chicken and pig liver after oral administration of phthalates (Jarosova et al., 1999).

In fattening pigs ( $n = 6$ ) and beef cattle ( $n = 6$ ), examined on farms in the Hodonin district in 1997 (Raszyk et al., 1998), the levels of DBP found in fat were higher than those of DEHP. The DBP and DEHP contents in backfat of pigs were 3.63 and 0.50 mg/kg of fresh sample, respectively. In the kidney fat, the contents of DBP and DEHP were 2.54 and 0.79 mg/kg of fresh sample. The ratio between DBP and DEHP was 7 : 1 in swine fat and 3 : 1 in cattle fat, while we found the ratio between DBP and DEHP in fat of 1 : 2 to 3 in the chicks we examined.

The results suggest that in the chicks mainly DEHP is accumulated in fat, whereas in pigs mainly DBP is accumulated. However, this assumption should be either confirmed or disconfirmed by examining much higher numbers of chicks, pigs and cattle.

Complete feed samples ( $n = 21$ ) collected in 1997 on swine, cattle and poultry farms (Raszyk et al., 1998) contained lower phthalate levels (DBP 0.21 mg/kg, DEHP 0.22 mg/kg of fresh sample) than the samples of complete feeds BR<sub>1</sub>, BR<sub>2</sub> and BR<sub>3</sub> used in the previous study (DBP 0.96 to 1.37, DEHP 0.48 to 0.76 mg/kg of fresh sample).

The last blanket monitoring of phthalates in raw materials and foodstuffs of animal origin in the Czech Republic was carried out in 1996 and 1997. In several State Veterinary Institutes, five

samples of fresh meat, 33 samples of meat products, 29 samples of poultry meat and insides, 28 samples of market milk and 22 samples of milk products were examined. Mean levels of a sum of phthalates (DEHP + DBP) in the above mentioned samples were as follows (mg/kg of fresh sample): fresh meat 1.28 mg/kg, meat products 1.07 mg/kg; poultry meat and insides 0.67 mg/kg; market milk 0.27 mg/kg, milk products 0.31 mg/kg

The control chicks (K) showed the content of a sum of phthalates (DBP + DEHP) in the muscle of 0.57 mg/kg of fresh sample, i.e. a little less than in poultry meat and insides (0.67 mg/kg) in 1996 and 1997.

The most commonly reported target organs, damaged by acute toxicity, are the testicles and liver. The most frequent alterations are atrophy of testicles and hepatomegaly (David et al., 2000). In pigs, DEHP influences the function of bulbourethral glands (Ljungvall et al., 2003). During subacute toxicity, a proliferation of peroxisomes develops (Ortiz-Zarragoitia et al., 2006), and the induction of peroxisomal enzymes, changes in the lipid metabolism, inhibition of cholesterol synthesis and induction of fatty acid beta-oxidation may develop. During chronic toxicity (a long-term exposure *in vivo* in rats or mice, *in vitro* in tissue cultures), the following undesirable effects of PAE were demonstrated: teratogenicity and embryotoxicity, spermiotoxicity, nephrotoxicity, hepatotoxicity, carcinogenicity and adverse effects on membrane functions (Marhold, 1980).

The current legislation effective in the Czech Republic does not cover the issues related to phthalates in foodstuffs and farm animal feedstuffs. Czech law, effective till 2004, defined chemical requirements for the health safety of different kinds of foodstuffs and food raw materials. Admissible levels of phthalates in muscle and of farm animals (expressed as a sum of DEHP and DBP) of 2 mg/kg 4 mg/kg of fresh sample, respectively, was defined.

In the Czech Republic, hygiene limits for contents of phthalates in farm animal feeds have not been elaborated yet, therefore these preliminary results may serve as basis for solving this problem.

Czech legislation on hygiene requirements for toys and products for children till three years of age stipulates that toys made of softened plastics must not contain more than 0.1% phthalates on mass basis.

Czech legislation on hygiene requirements for products intended for contact with foodstuffs and

dishes stipulates that plastics and products from plastics intended for contact with feedstuffs must not release their own components in feedstuffs in amounts higher than 10 mg per square decimetre of product surface, or the limit for total migration of released components is expressed as 60 mg per kg of a feedstuff or feedstuff-like substance.

On the basis of comprehensive knowledge on effects of phthalates on live organisms and data on the occurrence of phthalates in the environment and food chain it is necessary to adopt any measures to decrease the risk of PAE occurrence in the food chain. Because the levels of phthalic acid esters found in the food chain can be classified as hazardous according to the Regulation (EC) No. 178/2002 (Anonymous, 2002), feed business operators should include the control of this hazard in their HACCP systems according to the Regulation (EC) No. 183/2005 (Anonymous, 2005) on feed hygiene. At present there is not enough data on feed contamination with phthalates, therefore phthalate contents in the food chain should be further monitored and competent authorities should define legislatively maximum permitted concentrations in feedstuffs and feedstuffs (Harazim et al., 2008). In agreement with the opinion of Latini (2005) who stated that the environmental phthalate monitoring should continue and its maximum allowed concentrations should be determined by regulations, we recommend to monitor feedstuffs and feed ingredients, drinking and service water and feedstuffs for residues of DEHP and DBP; a suitable indicator of DEHP and DBP contamination in animals is the adipose tissue (subcutaneous fat in swine, kidney fat in cattle and poultry fat). Muller and Kordel (1993) in their experiment did not demonstrate the uptake of phthalates by plants from soil and expressed the opinion that an important route of phthalate uptake by plants can be the accumulation of phthalates by the plant cuticle from the air. For this reason, it is desirable to continue the monitoring of the route of uptake and the way of accumulation of phthalates in plants. The monitoring of phthalate contents in the stable environment on swine, cattle and poultry farms will allow us to assess the total exposure of animals to phthalates. In agricultural enterprises, feed manufacturing plants and food manufacturing plants, revisions of plastics should be carried out that are part of production technologies and structures, as well as revision of paints and other hazardous materials that come into contact with feedstuffs,

feedstuffs, raw materials or animals. Other things to be monitored include plastic packaging materials, barrels and tanks used for the storage of feedstuffs and foodstuffs, colour printings, adhesives, and other substances that come into contact with feedstuffs and foodstuffs. The sources of environmental contamination with phthalates should be mapped out (industrial waste water, incineration plants, landfills) that may subsequently contaminate sources for the foodchain in surroundings of farms, feed mills, water sources and food processing plants.

Gradually, toxic phthalates used as plasticizers and adhesives should be substituted with other, non-toxic, substances (citrate, phenol alkyl sulphate, benzoates), particularly in the production of materials used in agriculture, food industry and health service (Patrick, 2004).

The data on distribution and accumulation of DEHP and DBP in the body of farm animals after the oral administration of phthalates received in this study are important from the viewpoint of health safety of feedstuffs and foodstuffs and their protection from toxic phthalates. From the viewpoint of food hygiene, the finding that neither the chicks fed the high phthalate diet, nor the chicks fed the animal fat supplemented diet showed increased phthalate contents in the muscle as compared with the control chicks can be regarded as favourable finding.

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