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REGULAR ARTICLE

DI-2-ETHYLHEXYL PHTHALATE AND DI-N-BUTYL PHTHALATE IN TISSUES OF COMMON CARP (*Cyprinus Carpio L.*) AFTER HARVEST AND AFTER STORAGE IN FISH STORAGE TANKS

Alžbeta Jarošová, Lenka Puškárová, Vlasta Stancová*

Adress*: doc. Ing. Alžbeta Jarošová, Ph.D., Mendel University in Brno, Czech Republic, Department of Food Technology, Zemědělská 1, 613 00 Brno, phone number: +420 545 133 191; e–mail: ualja@mendelu.cz

ABSTRACT

The aim of the present study was to determine whether the influence of fish pond and fish storage tank conditions change the content of phthalic acid esters (di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP)) in the carcass of the Common carp. Samples obtained from the autumn harvest of two fish ponds (R1 and R2) in 2007 and 2010 from the South Moravia and after a seven-week-long storage in fish storage tanks were analyzed. It was found that in the samples (2007) from both fish ponds after storage in fish storage tanks the content of DBP increased, for R1 from 0.56±0.39 $mg.kg^{-1}$ to 0.83±0.26 $mg.kg^{-1}$ and for R2 from 0.31±0.08 $mg.kg^{-1}$ to 0.35± 0.11 $mg.kg^{-1}$of original weight. Concerning DEHP, in samples obtained from the fish pond R1 after storage in fish storage tanks the mean concentration increased from 0.26±0.23 $mg.kg^{-1}$ to 0.72±0.16 $mg.kg^{-1}$ and from R2 it decreased from 0.71±0.17 $mg.kg^{-1}$ to 0.12±0.18 $mg.kg^{-1}$ of original weight. The experiment conducted in 2010 should have confirmed or disconfirm this trend. The average value of the DBP contents for R1 increased slightly from 0.15±0.04 $mg.kg^{-1}$ after harvest to 0.18±0.05 $mg.kg^{-1}$ of original weight after the storage in fish storage tank and for R2 from 0.09±0.03 $mg.kg^{-1}$ after harvest to 0.16±0.06 $mg.kg^{-1}$ of original weight after storage in fish storage tank. Similarly, an increasing tendency also occurred for DEHP, for R1 the value of 0.08±0.02 $mg.kg^{-1}$ after harvesting increased to 0.14±0.04 $mg.kg^{-1}$ of original weight after storage in fish storage tank and for R2 from 0.09±0.03 $mg.kg^{-1}$ after harvesting to 0.16±0.06 $mg.kg^{-1}$

after storage in fish storage tank. For all samples in both lakes in 2007 as well as in 2010 both phthalates showed slightly increasing trend except for DEHP in samples collected in 2007 from the fish pond R2.

Keywords: Phthalates, di-n-butyl phthalate, di-2-hexyl phthalate, fish

INTRODUCTION

Phthalic acid esters (PAE) are a group of foreign contaminating substances which, owing to its excellent plasticizing properties and adhesion, find extensive application in all areas of human activity **(Cano et al., 2002).** For a long-term exposure, their adverse effects on living organism have been demonstrated **(Matsumoto et al., 2008, Casas et al., (2011)**. Chronic intake of low concentrations of phthalates can lead to many health problems **(Norman et al., 2007)**.

Population can be exposed to them through inhalation **Bornehag et al. (2004),** oral **(Schettler, 2006)**, and skin resorption **(Api, 2001)**.

The main and possibly only source of phthalates is human activity. The release of phthalates into the environment occurs not only in their production **(Clausen et al., 2004)** and manufacture of materials containing phthalates, but also during their use and subsequent disposal. Waste water, atmosphere, and soil can be contaminated **(Zheng et al., 2007)**. The main source of soil contamination are industrial and municipal wastes. In addition, phthalates can get into the soil after application of agricultural products and as a result of air emissions **(Velíšek, 2002)**.

As the phthalates generally occur in the aquatic environment, it is likely that fish are also exposed to phthalates through aqueous column, food, and sediment **(Peijnenburg and Struijs, 2006)**.

Stalling et al. (1973) analyzed DEHP in fish from various regions of the United States of America. They found residues ranging from 0.2 to 10 $\mu g.g^{-1}$ of the original sample. The largest concentrations of residues were from fish originating from industrial areas. DBP was metabolized by liver microsomes of catfish in vitro sixteen times faster than DEHP.

Huang et al. (2008) observed some amount of phthalic acid esters in fish in 17 Taiwanese rivers. They found the highest concentrations of DEHP in the species of Liza subviridis, exactly 253.9 $mg.kg^{-1}$ of dry matter and in the species of Oreochromis niloticus

niloticus, exactly 129.5 $mg.kg^{-1}$ of dry matter. Their research confirmed that DEHP contamination in fish is affected by habitat and physico-chemical properties of contaminants.

The European Community does not set limits for phthalates in food. The *EC Scientific Committee on Food* has set tolerable daily intake (TDI) of dibutyl phthalate at 0.050 mg per kg of live weight and di-2-ethylhexyl phthalate at 0.025 mg per kg of live weight. The established value of tolerable daily intake (TDI) for DEHP is 50 $\mu g.kg^{-1}$ of body weight, the actual intake of phthalates from food is estimated at 0.15 to 0.3 mg/person/day, which is the limiting value for the TDI **(Velíšek, 2002**).

In this work, the authors aimed to determine whether fish pond and fish storage tank conditions will have an influence on the content of the most common phthalates (di-n-butyl phthalate and di-ethylhexyl phthalate) in body of the Common carp (*Cyprinus carpio*).

MATERIAL AND METHODS

Material

Samples were obtained from carp harvest of two fish ponds (R1 and R2) in South Moravia. In the autumn harvest, 10 samples of the Common carp were randomly selected from each fish pond. Subsequently, fish were placed in fish storage tanks, thus they were temporarily stored here until the time of sale. Similarly, samples were taken from fish (n = 10) after their storage in fish storage tanks (after about seven weeks). After being killed, the carps were gutted and stored at -21 °C. Muscle with skin from the right half was used for chemical analysis. Samples were processed in the laboratory of the Department of Zoology, Fisheries, and Hydrobiology of Mendel University in Brno and preparation of samples and their chemical analysis was performed at the Department of Food Technology of Mendel University in Brno.

Harvesting and transporting fish into fish storage tanks took place in the months of November to December in 2007 and 2010.

Methods Applied

Tissue samples were analyzed immediately after thawing at refrigerator temperatures. Established methods for the determination of phthalates in food were used to determine the PAE **(Jarošová et al., 1998 and 1999)**.

Homogenized samples of fish muscle with skin were lyophilized and subsequently residues of PAE were extracted using a solvent mixture of acetone:hexane (1:1). PAE were separated from co-extracts by gel permeation chromatography employing gel of Bio beads S-X3. The cleaning procedure with concentrated sulfuric acid was used for final purification of the eluate. Determination of PAE was carried out using high-performance liquid chromatography (HPLC) with UV detection at 224 nm. The column used was Zorbax Eclipse C8, 5 µm, 4.6 mm × 150 mm, mobile phase was acetonitrile:water (99:1). Evaluation was performed utilizing the Agilent chemstation software.

All samples were analyzed in duplicate. Concentrations of DEHP and DBP are related to the original sample. In total 80 samples were analyzed.

The results were processed using the statistical program of STATISTIKA 9. T-test and Duncan's test were used.

RESULTS AND DISCUSSION

Results Obtained in 2007

Concerning the fish pond R1 (Tab 1), mean DBP value of samples from the autumn harvest was 0.56±0.39 $mg.kg^{-1}$ of original weight and after a 7-week-long storage in fish storage tank it increased to 0.83±0.26 $mg.kg^{-1}$ of original weight. Concerning the fish pond R2, mean content of DBP was slightly lower (0.31±0.08 $mg.kg^{-1}$ of original weight), but the tendency in relation to storage in fish storage tanks repeated as for R1, though the increase of mean concentration was significantly lower (0.35±0.11 $mg.kg^{-1}$ of original weight) than for R1. Differences in DBP concentrations in carps from the autumn harvest and after the storage in fish storage tanks were not significant for any fish pond ($p>0.05$), yet at R1 as well as R2 after the storage in fish storage tank an increase in DBP contents occurred. According to **Zeng et al. (2008)** DBP is the most represented phthalate in the fish pond waters.

The average content of DEHP in samples from the fish pond R1 was 0.26±0.23 $mg.kg^{-1}$ of original weight and, after storage in fish storage tank, the measured valueswere again higher, as with DBP, the mean value represented 0.72±0.16 $mg.kg^{-1}$ of original weight. Differences in concentrations of DEHP in carps from the autumn harvest and after storage in fish storage tank were statistically significantly different ($p<0.05$).

Another situation was found at the autumn harvest with DEHP in samples from the fish pond R2, where the average value was significantly higher (0.71±0.17 $mg.kg^{-1}$) than in

samples from the fish pond R1 (0.26±0.23 mg.kg^{-1}). There was a decrease of 0.71±0.17 mg.kg^{-1} after harvest to 0.12±0.18 mg.kg^{-1} of original weight after the storage in fish storage tank, the differences in concentrations were statistically different (p>0.05).

Tab 1 Statistical characteristics (n = number of samples, mean, median, SD = standard deviation, min. and maximum value), DBP, DEHP and sums of DBP and DEHP (mg.kg^{-1} of original weight) of carp muscle samples obtained from the autumn harvest in two fish ponds (R1 and R2) and after storage in fish storage tanks in 2007

	Autumn Harvest						**Storage in Fish Storage Tank**					
	DBP		**DEHP**		**Σ DBP+DEHP**		**DBP**		**DEHP**		**Σ DBP+DEHP**	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
	[mg.kg^{-1} of original weight]											
n	10	10	10	10	10	10	10	10	10	10	10	10
Diameter	0.56	0.31	0.26	0.71	0.82	1.02	0.83	0.35	0.72	0.12	1.55	0.46
Median	0.38	0.31	0.19	0.74	0.65	1.05	0.84	0.33	0.77	0.05	1.45	0.38
SD	0.39	0.08	0.23	0.17	0.51	0.20	0.26	0.11	0.16	0.18	0.37	0.23
Min.	0.20	0.20	0.04	0.46	0.29	0.74	0.37	0.22	0.35	0.03	0.86	0.22
Max.	1.53	0.46	0.62	0.96	1.93	1.36	1.29	0.60	0.96	0.66	2.10	0.98

The overall mean concentration of DBP and DEHP in samples from the fish pond R1 was 0.82±0.51 mg·kg^{-1} for the autumn harvest and 1.55±0.37 mg.kg^{-1} of original weight after storage in fish storage tank. Concerning the fish pond R2, the sum of both phthalates concentrations (DBP and DEHP) had a decreasing trend (p>0.05), there was observed a trend of higher concentrations noticed at DEHP. Here, the average concentration of 1.02±0.20 mg.kg^{-1} decreased to 0.46±0.23 mg.kg^{-1} of original weight.

Results Obtained in 2010

Tab 2 Statistical characteristics (n = number of samples, mean, median, SD = standard deviation, min. and maximum value), DBP, DEHP and sums of DBP and DEHP (mg.kg^{-1} of original weight) of carp muscle samples obtained from the autumn harvest in two fish ponds (R1 and R2) and after storage in fish storage tanks in 2010

	Autumn Harvest						**Storage in Fish Storage Tank**					
	DBP		**DEHP**		**Σ DBP+DEHP**		**DBP**		**DEHP**		**Σ DBP+DEHP**	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
	[mg.kg^{-1} of original weight]											
n	10	10	10	10	10	10	10	10	10	10	10	10
Diameter	0.15	0.09	0.08	0.09	0.23	0.18	0.18	0.16	0.14	0.16	0.32	0.32
Median	0.14	0.10	0.08	0.10	0.21	0.20	0.17	0.15	0.14	0.15	0.32	0.30
SD	0.04	0.03	0.02	0.03	0.05	0.05	0.05	0.06	0.04	0.06	0.08	0.12
Min.	0.10	0.04	0.05	0.04	0.15	0.08	0.07	0.07	0.09	0.05	0.17	0.12
Max.	0.24	0.12	0.11	0.12	0.24	0.2	0.25	0.28	0.20	0.28	0.45	0.55

Concerning the fish pond R1 (Table 2), when mean DBP concentration before the storage in fish storage tank (0.15±0.04 mg.kg^{-1} of original weight) is compared to the value after the storage in fish storage tank (0.18±0.05 mg.kg^{-1} of original weight), one can notice increased mean concentrations in samples of muscle tissue ($p>0.05$). When mean DEHP concentration before the storage in fish storage tank (0.08±0.02 mg.kg^{-1} of original weight) is compared to the value after the storage in fish storage tank (0.14±0.04 mg.kg^{-1} of original weight), one can notice increased mean concentrations ($p<0.01$). The mean concentration of the sum of both phthalates in samples of muscle tissue after autumn fish pond harvest was 0.23±0.05 mg.kg^{-1} of original weight, after the storage in fish storage tank 0.32±0.12 mg.kg^{-1} of original weight.

When mean DBP concentration from the fish pond R2 before the storage in fish storage tank (0.09±0.03 mg.kg^{-1} of original weight) is compared to the value after the storage in fish storage tank (0.16±0.06 mg.kg^{-1} of original weight), one can notice an increased mean concentration in muscle tissue samples ($p<0.01$). When mean DEHP concentration before the storage in fish storage tank (0.09±0.03 mg.kg^{-1} of original weight) is compared to the value

after the storage in fish storage tank (0.16±0.06 mg.kg^{-1} of original weight), one can notice an increased mean concentration ($p<0.01$). The mean concentration of the sum of both phthalates in samples of muscle tissue after autumn fish-pond-R2 harvest was 0.18±0.05 mg.kg^{-1} of original weight, after the storage in fish storage tank 0.32±0.12 mg.kg^{-1} of original weight.

The assumption that PAE content decreases in the muscle tissue of fish after their storage in fish storage tanks, where they do not consume food any more, that is one of the possible sources contamination PAEs, was not confirmed. Concerning the fish pond R1 in 2007 as well as in 2010 (it is the same fish pond in both years), in the experiment after the storage in fish storage tank a slight increase in the concentration of DBP, DEHP and the sum of both phthalates occurred. For samples from the fish pond R2 in 2007, this increasing trend was observed after the storage in fish storage tank only for DBP. The average concentration of DEHP after harvest was significantly higher (0.71±0.17 mg.kg^{-1}) and after storage in fish storage tank it decreased (0.12±0.18 mg.kg^{-1}). Samples analyzed in 2010 for R2 showed after storage in fish storage tank an increasing trend.

We can conclude that the detected amounts of DBP and DEHP get into fish tissue from the natural environment, i.e. from water and sediments, also confirmed **Bauer and Herrmann (1997) and Bauer et al. (1998). Yuan et al. (2002)** examined the representation of 8 types of phthalates in aquatic sediments and demonstrated the greatest incidence of DEHP and DBP.

In a study by **Adeniyi et al. (2011)** DMP was neither detected in samples of water nor fish, but in the sediment samples. Concentrations of DEP, DBP and DEHP in fish ranged from 0.32−0.81; 0.38−1.08, and 0.04−0.15 mg.kg^{-1} for Oreochromis niloticus niloticus, 0.31−0.86; 0.40−1.170 and 0.04−0.11 mg.kg^{-1} for Mystus, and 0.32−0.81; 0.40−3.97 and 0.03−0.30 mg.kg^{-1} for Synodontis. Differences in concentrations of phthalates in fish were not statistically significant ($p>0.05$).

CONCLUSION

In order to detect the contents of PAE, muscle samples of the Common carp collected from two fish ponds (R1 and R2) in South Moravia at the autumn harvest and after their storage in fish ponds and fish storage ponds in 2007 and 2010 were analyzed to determine whether the storage in fish storage tank affects the content of PAE.

In samples obtained from the fish pond R2 in 2007, as a result of storage in fish storage tank, in DBP at R1 and R2 and DEHP at R2 occurred a slight increase in the

concentration of phthalate, and in DEHP at R2 occurred a drop in phthalate content. The resulting sum of both phthalates reflected the trend observed for DEHP, and even here at R2 was found a decrease in concentration after storage in fish storage tank.
In the 2010 experiment after the storage in fish storage tank there was a slight increase in the concentration of DBP, DEHP, and the sum of both phthalates.

Differences in measured values have an obvious cause, although it was from 2 Moravian fish ponds, each of them is fed by another river. For the level of phthalate contamination in rivers and water bodies it is critical, whether it lies in an industrial zone or not. Other factors could be soil, dredging or level of biodegradation processes in individual fish ponds.

Based on the results, we can conclude that while the storage in fish storage tank was accompanied by an increase in concentration of phthalates, these elevated values do not pose a health risk to consumers. Average concentrations of DEHP and DBP ranged mostly in tenths of $mg.kg^{-1}$ and the sum of concentrations of DEHP and DBP in any of the samples did not exceed legislative limit (4 $mg.kg^{-1}$) for fish which was in force earlier. Concentrations of phthalates could rise as a result of industrial processing, packaging, storage, or preparation.

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