Uptake, Excreation, and Metabolism of ^{14}C-labelled Di-2-ethylhexyl phthalate by Mullet, *Mugil cephalus*

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Mulletts, *Mugil cephalus* were exposed to artificial sea water containing 50$\mu g/l$ of ^{14}C-labelled di-2-ethylhexyl phthalate(DEHP) during 15 days and returned to the DEHP free sea water in order to know bioconcentration and depuration of DEHP in the fish.

Bioaccumulative process of DEHP in the fish was rather fast, and bioconcentration level of 9.7~14$\mu g/g$ and a bioconcentration factor of 220~270 were reached after one day of exposure. The biological half-life of DEHP in fish was 1.8 days.

Five intermediate metabolites of DEHP were detected in the benzene and ethyl acetate fraction of fish by TLC.

KEY WORDS: mullet, ^{14}C-di-2-ethylhexyl phthalate (^{14}C-DEHP), bioconcentration factor, biological half-life, metabolism.

Introduction

Phthalate esters are widly used in plastic industry as a flexibilizer for synthetic polymers. Among them, di-2-ethylhexyl phthalate(DEHP) is the most important flexibilizer for polyvinyl chloride(PVC) and it constitute about 40% of the end products of PVC in weight (Marx, 1972).

As a result of extensive use of PVC in various products such as construction materials, interiors of vehicles, medical products, and packing materials for food, DEHP is commonly found in our environment (Mayer *et al.*, 1972). The accumulation and metabolism of DEHP are rather fast (Brown and Thompson, 1982; Sanders *et al.*, 1973; Mayer, 1976) and produce toxic effects on the reproduction of aquatic organisms (Mayer and Sanders, 1973; Sanders *et al.*, 1973).

Therefore, the evidence that phthalates can become environmental contaminants and exert harmful effects on some invertebrates and fishes. Hence, a study of the structure-bioaccumulation-toxicity relationship would be useful for elucidation of the effect of toxicity on aquatic life.

The present study was conducted to understand the bioaccumulation, excreation and metabolic features of DEHP in mullet.

Materials and Methods

Test solution and supply system

Twenty five *mg* of DEHP and 500μg of ^{14}C-carbonyl-labelled DEHP (Daiichi Pure Chemicals Co., LTD, specific activity; 146$\mu Ci/mg$) (Fig. 1) were emulsified with Tween-60 and HCO-40 under ultrasonic irradiation for 30 minutes at 80℃. The dispersed solution containing 50mg/l of DEHP were stored in the refrigerator as stock solution. The solution were pumped to the mixing chamber and di-

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luted to 50*μg/l* with 17‰ artificial sea water and supplied to the test vessel (Fig. 2). Water temperature was held at 25± 2℃ during the experiment using a submerged heater. The radioactivity of the stock solution and the test solution were monitored with a liquid scintillation counter (Mark III, Searle Analytic Inc.) to maintain the homogeneity of the solutions throughout the study period.

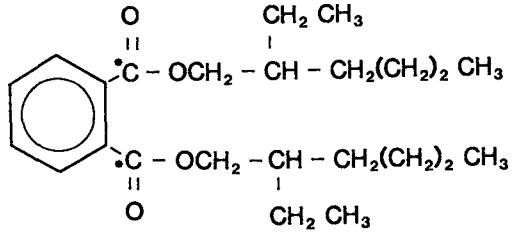

Fig. 1. Molecular structures of ^{14}C-di-2-ethylhexyl phthalate (*: ^{14}C -labeled position).

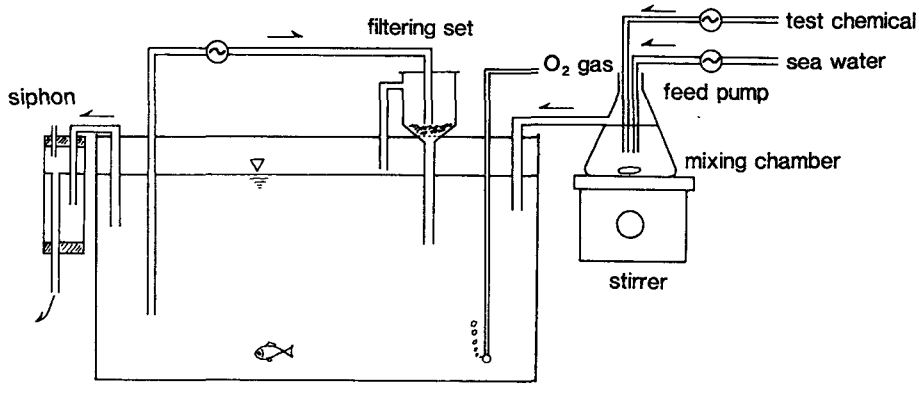

Fig. 2. A schematic diagram of the continuous flow water system of bioconcentration test.

Fish

Two hundred mulletts were acclimatized in 40*l* a glass tank filled with 17‰ artificial sea water during one month. After acclimatization, one hundred healthy and even size of fish were chosen for the each series of experiments. Mean body weight and mean body length of the fish were 4.3± 0.4*g* and 1.7± 0.4*cm*, respectively. The fish fed a commerical diet daily with 2~4% of body weight.

Test procedure

As subsamples, 5 fishes were collected randomly at time intervals of 6 hours, 1 day, 3 days, 5 days, 7 days and 14 days. The fishes were dried at 60℃ for 24 hours and pulverized. The to 20*mg* of the fish powder was oxidized in an automatic sample oxidizer (Packard Model 306) and the radioactivity was measured by LSC.

In order to determine excretion rate of DEHP, 50 fishes were transferred to DEHP-free artificial sea water. Subsamplings and radioactivity analysis were conducted same as above.

The intermediate metabolites of DEHP were extracted from the whole body of fish with benzene-ethyl acetate (1: 10, v/v) and 20% H_2SO_4 using a vortex homogenizer. The authenic spots on the TLC plate were detected using a fluorescent lamp. The extracts were placed on a silica gel plate (Merk, Silica Gel 60 F-254) after adjusting the pH to neutral and developed in benzene-ethyl acetate (6: 1, v/v) as an eluent (Fig. 3). Silica gels on the TLC plate were scraped off for every 10*mm* from the original point to the solvent front, and the distribution of radioactivities were measured.

Results and Discussion

Bioaccumulation of ^{14}C-DEHP in mullet was quite rapid and the concentration value reached to plateau levels by the first day of exposure (Fig. 4). Thereafter, the equilibrium level on the concentration-time curve showed little changes. The maximum concentration value of 14*μg/g* was found after

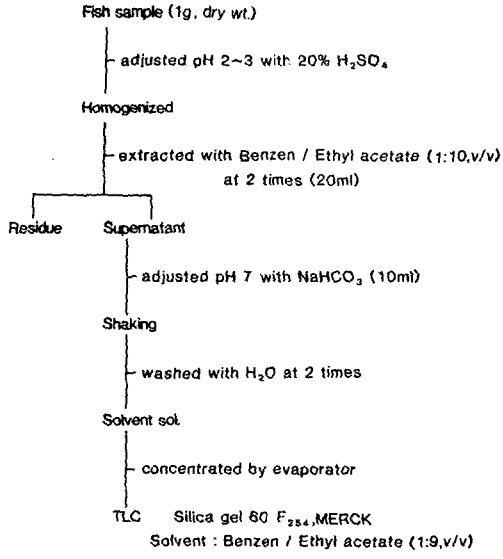

Fig. 3. Preparation procedures of ^{14}C-DEHP metabolites in mullet by TLC.

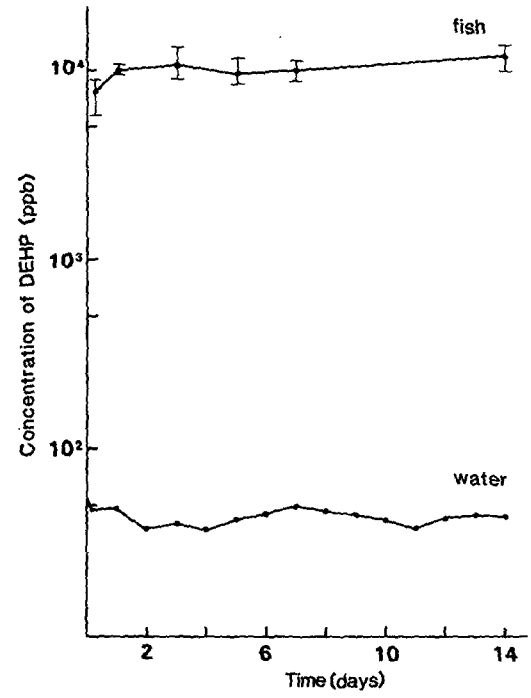

Fig. 4. Uptake of ^{14}C-DEHP by mullet in 50 ppb DEHP continuous flow water system.

14 days of the experiment, however, considering little fluctuations on the concentration-time curve, it seemed that the mean of 1, 3, 5 and 7 days concentration was more close to the real equilibrium concentration. The plateau level concentration and bioconcentration factor (BCF) were appeared to be 9.7~14$\mu g/g$ and 220~270, respectively.

The BCF of the present study was similar to the results of Mayer and Sanders (1973) and Brown and Thompson (1982a) who studied *Daphnia magna*. Macek *et al.*(1979) also found that the equilibrium concentration of DEHP reached within 24 hours in *D. magna*. On the other hand, Brown and Thompson (1982b) found somewhat higher BCF of 2,500~3,500 in mussel, *Mytilus edulis* and Mayer (1976) reported that the BCF ranged downward from 886 to 155 as the exposure concentrations increased in fathead minnows, *Pimephales promelas* which were continuously exposed to 1.9~62$\mu g/g$ DEHP for 56 days. The BCF are now commonly being used in assessing propensity of a chemical to accumulate in aquatic organisms, with little consideration of the concentration on the surrounding medium. Therefore, BCF changes depend on the concentration of chemical surrounding the organisms.

The depuration of DEHP from the fish body to surrounding environment was also rapid. More than 90% of DEHP was lost within 10 days in DEHP-free artificial sea water, and the biological half-life was appeared to be 1.8 days (Fig. 5). The biological hald-life of in fathead minnows was 12.2 days (Mayer, 1976) and 3.5 days in mussel (Brown and Thompson, 1982b).

To examine the metabolism of DEHP in fish. TLC was conducted with benzene-ethyl acetate soluble fractions of fish bodies. Six spots were detected. Of these, A(Rf value, 0.79), B(0.75), D(0.65), E(0.1) and F(origin) were intermediate metabolites, and C(0.69) was DEHP (Fig. 6).

The proportion of DEHP in the fish body decreased with time. About 20% of DEHP was metabolized within 6 hours and 55% was metabolized within 7 days (Fig. 7). As compared to the slow metabolic rate of ^{14}C-DEHP in the fish body and its biological half-life, it seemed that the most of DEHP may be excreated as the orignal form. On the other hand, Wofford *et al.*(1981) reported that penaeid shrimp, *Penaeus aztecus* had a higher metabolic capacity of esters, and sheepshead minnows,

Cyprinodon variegatus were the most efficient at converting the phthalate esters to their more polar metabolites. Mayer (1976) found that the fathead minnows degraded DEHP to 2-ethylhexyl phthalate and phthalic acid. Studies on the *in vivo* metabolism of DEHP in rats (Albro *et al.*, 1973; and Albro and Moore, 1974) showed that DEHP was predominantly metabolized by hydrolysis of one ester bond and both terminal(ω) and subterminal(ω-1) oxidation of the remaining alkyl chain. Marx (19 72) reviewed that some phthalates are extremely toxic to replicating mouse fibroblasts in cell culture, and dimethyl phthalate and DMEP(dimethoxyethyl phthalate) markedly inhibited the reproduction of such cells.

Further investigations that isolated fractions on the TLC plate will be subjected to GC-MS analysis.

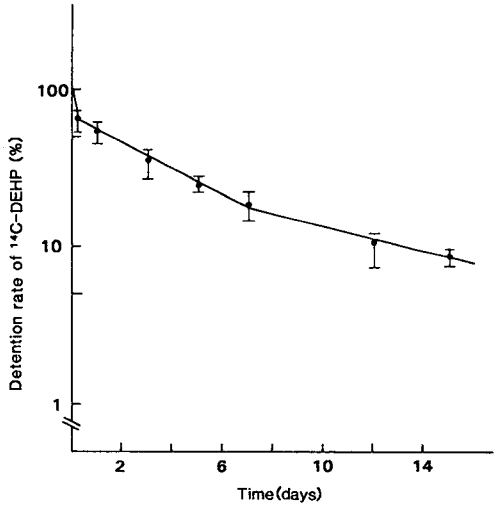

Fig. 5. Detention rate of ^{14}C-DEHP by mullet.

Acknowledgements

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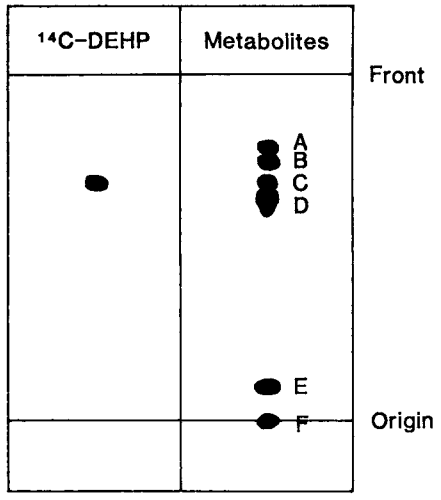
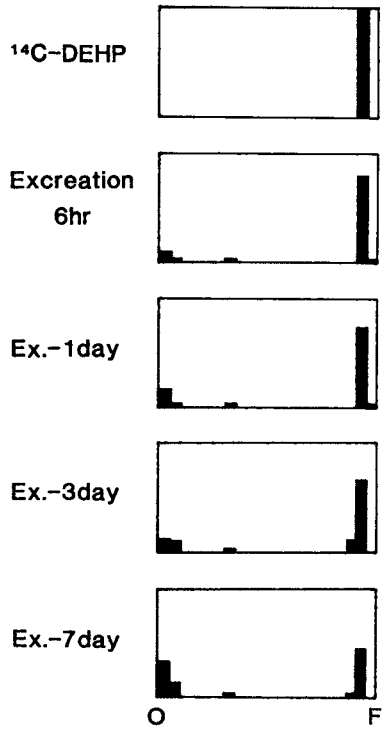


solvent: Benzen / Ethyl acetate (1/9 , v/v)
TLC(Silica gel 60 F_{254}, Merck)

Fig. 6. Thin layer chromatography of ^{14}C-DEHP and their metabolites in mullet, ^{14}C-DEHP Rf: 0.69, A: 0.79, B: 0.75, C: 0.69, D: 0.65, E: 0.1, F: origin.



TLC(silica gel 60 F_{254}, Merck)
Solvent: Benzen / Ethyl acetate (1/9, v/v)

Fig. 7. Changes of intermediate metabolites of ^{14}C-DEHP by mullet.

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