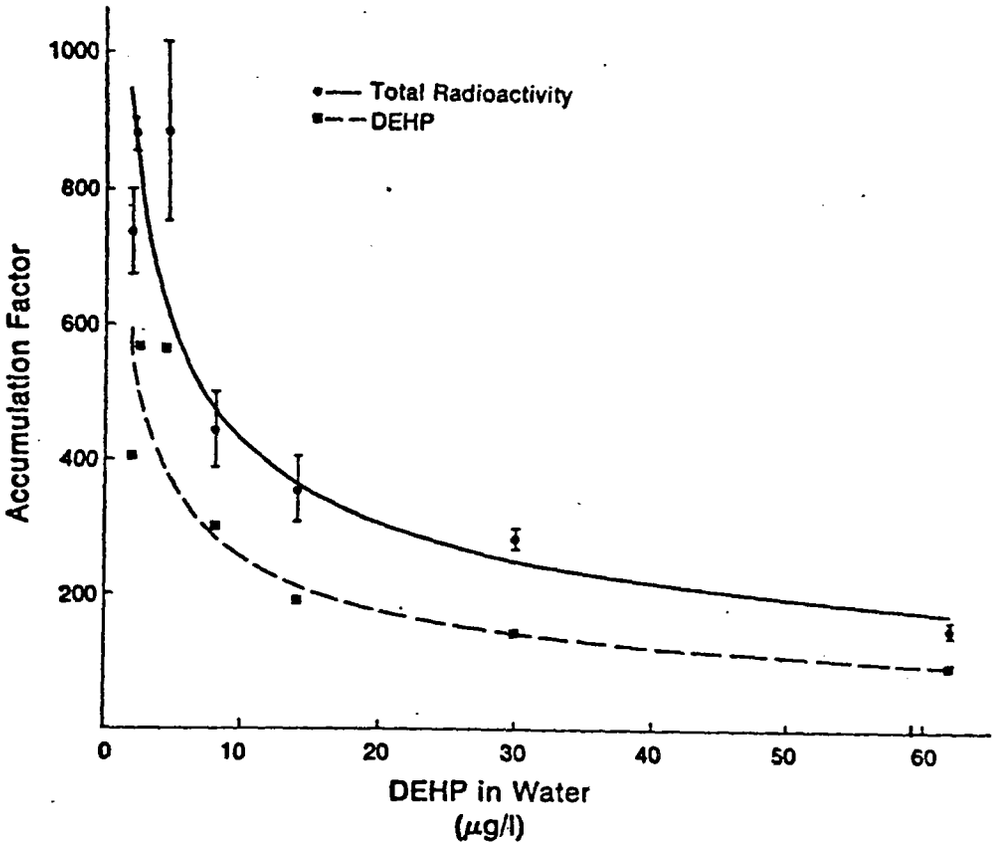

Fig. 1. Effect of concentration on the accumulation of ^{14}C carbonyl-labeled di-2-ethylhexyl phthalate in fathead minnows after 56 days of exposure. (Total radioactivity=mean and standard error for four fish; DEHP=gas chromatographic value of DEHP only in a pooled sample of four fish).

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2. Percentage compositon of ^{14}C labeled residues in fathead minnows after 56 days of continuous exposure to ^{14}C carbonyl-labeled di-2-ethylhexyl phthalate.

Exposure concentration (µg/l)	Residue					
	DEHP[a]	MEHP[b]	Phthalic acid	MEHP conjugate[c]	Phthalic acid conjugate[c]	Other[d]
1.9	79	12	2.5	4.1	1.2	1.2
2.5	70	24	1.7	2.2	1.1	1.0
4.6	69	23	4.1	1.2	0.6	2.1
8.1	70	21	5.4	1.0	1.2	1.4
14	60	30	3.6	0.6	5.1	0.7
30	42	40	11	0.4	6.6	0.3
62	33	50	5.7	0.4	9.8	1.1

[a] Di-2-ethylhexyl phthalate

[b] 2-ethylhexyl phthalate

[c] Glucuronid conjugates of MEHP and phthalic acid

[d] Unidentified material remaining at origin during thin layer chromatography

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Table 1. Total ^{14}C carbonyl-labeled di-2-ethylhexyl phthalate (ug/g) in Fathead minnows held at 25 C during periods of uptake (days 1-56) and elimination (days 57-84); standard errors in parentheses.

Days	Mean exposure concentration[a] (ug/l)						
	1.9 (0.1)	2.5 (0.1)	4.6 (0.2)	8.1 (0.2)	14 (0.6)	30 (0.7)	62 (3)
1	0.26 (0.02)	0.40 (0.05)	0.48 (0.04)	1.2 (0.1)	1.4 (0.1)	1.8 (0.1)	2.1 (0.1)
3	0.46 (0.09)	0.77 (0.08)	1.0 (0.1)	1.8 (0.1)	2.6 (0.1)	3.4 (0.1)	3.1 (0.7)
7	0.70 (0.07)	0.93 (0.12)	2.2 (0.5)	2.9 (0.5)	3.3 (0.2)	3.2 (0.3)	3.9 (0.1)
14	0.87 (0.12)	1.5 (0.2)	2.6 (0.2)	2.8 (0.2)	2.7 (0.2)	3.2 (0.4)	3.8 (0.6)
28	1.4 (0.2)	2.2 (0.1)	3.1 (0.5)	2.9 (0.4)	4.8 (0.6)	7.5 (0.9)	6.9 (1.9)
56	1.4 (0.1)	2.2 (0.1)	4.1 (0.6)	3.6 (0.4)	5.0 (0.7)	8.6 (0.5)	9.6 (0.6)
63	0.73 (0.29)	0.95 (0.02)	1.9 (0.2)	1.9 (0.1)	2.7 (0.4)	3.2 (0.7)	9.6 (1.4)
70	0.38 (0.11)	0.64 (0.10)	1.1 (0.1)	1.3 (0.3)	1.1 (0.1)	1.7 (0.2)	7.8 (0.6)
84	0.32 (0.06)	0.28 (0.05)	0.54 (0.04)	0.73 (0.12)	1.2 (0.1)	1.8 (0.3)	4.2 (0.6)
Half-life (days)							
^{14}C	13.6	9.7	10.0	12.6	14.7	12.8	21.6
DEHP	14.2	6.2	6.2	11.8	13.3	15.4	18.3

[a]No background residues of DEHP were found in the control water or fish.

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a later increase in DEHP degradation and elimination, and therefore a lower accumulation factor. This hypothesis is supported by the composition of the ^{14}C-residues at 56 days (Table 2). As exposure concentrations increased, the proportion of DEHP decreased and degradation products of the diester increased. However, half-life determinations did not substantiate the hypothesis in its entirety (Table 1). The decrease in half-life with increasing concentrations through the 4.6 µg/liter exposure, followed by an increase at higher concentrations, possibly indicated liver malfunction after 56 days of continuous exposure (liver tissues were not examined).

Although DEHP accumulated in fish, the factor of accumulation was about 1% of that observed for DDT (Metcalf et al. 1973). Fathead minnows readily degraded DEHP to 2-ethylhexyl phthalate and phthalic acid, and the half-life was relatively short, averaging 12.2 days. The disappearance of DEHP residues after the fish were placed in uncontaminated water was due mainly to degradation and elimination and not dilution resulting from fish growth. The fish at the end of the elimination period weighed 33% more than those at the beginning (day 56), and the observed DEHP concentrations at the end were 17 to 58% of the calculated concentrations that would have been observed if the total decrease in DEHP had been from growth. However, the presence of DEHP residues is not without biological effect. Water concentrations of DEHP as low as 3 µg/liter have caused a 60% reduction in reproduction of Daphnia magna (Mayer and Sanders 1973; Sanders et al. 1973). The effects of DEHP or its degradation products on growth and reproduction in fishes are not yet known.

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was determined by the method of Chase and Rabinowitz (1967) and the effect of concentration on accumulation factors was analyzed by linear regression ($\log Y = a + b \log X$).

Results and discussion - The fish accumulated 1.4 and 9.6 ug/g of DEHP residues after 56 days of exposure in the 1.9 and 62 ug/liter concentrations, respectively (Table 1). The accumulation factors of 34 to 137 times the water concentration observed after 24 h exposure in fathead minnows agree well with the factors of 35 to 92 times observed by Metcalf et al. (1973) in the guppy (Lebistes reticulatus). Equilibria of ^{14}C-DEHP in fathead minnows were approached between 28 and 56 days of exposure. From the predicted equilibrium concentrations (1.5, 2.3, 3.7, 3.2, 5.2, 9.8, and 9.2 µg/g), residues in fish exposed to 1.9 and 2.5 µg/liter approached equilibria at 28 days, 4.6 and 8.1 µg/liter at 42 days, and 14, 30, and 62 µg/liter at 56 days.

Accumulation factors at 56 days ranged from 155 to 886 for total ^{14}C, and 91 to 569 for DEHP (Fig. 1), and decreased as exposure concentrations increased. Accumulation factors are now commonly being used in assessing the propensity of a chemical to accumulate in aquatic organisms, with little or no consideration of the effects of concentration in the surrounding medium. The present data indicate that large errors in deriving accumulation factors can occur if the organisms are not exposed to two concentrations having a range greater than one log interval.

Reductions in accumulation factors accompanying increases in exposure concentrations may have been due to the induction of detoxifying enzymes in the liver as reported in Hodgson (1968), and

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with non-labeled DEHP were prepared in acetone to give a specific activity of 180 cpm/µg. The amount of acetone did not exceed 0.25 ml/liter in the exposure vessels. DEHP concentrations in the exposure water were monitored radiometrically at weekly intervals by extracting 1-liter water samples with diethyl ether.

Sixty fathead minnows, 7.5 months old and each weighing 1.24 g (± SD 0.31) were placed in each of eight aquaria; seven of the lots were continuously exposed to different concentrations of DEHP for 56 days. After the exposure, the fish were placed in uncontaminated water for an additional 28 days. The fish were maintained, in general, according to the methods described by Brauhn and Schoettger (1975), and fed a commercial trout starter (EWOS) throughout the study.

Four fish were removed from each concentration and from the control for total radioactivity determinations after 1, 3, 7, 14, 28, and 56 days of exposure and after 7, 14, and 28 days in uncontaminated water. Total radioactivity in each fish was determined after Rodgers and Stalling (1972). DEHP and its degradation products were analyzed by thin layer chromatography (Stalling et al. 1973) on pooled samples of four fish each after 28 and 56 days of exposure and after 7, 14, and 28 days in uncontaminated water. In addition, DEHP was determined in each of the pooled samples by gas chromatographic analyses (Stalling et al. 1973).

Accumulation factors were determined by dividing the concentration in the fish (µg/g, wet weight) by the concentration in the water (µg/l) times 1000. Predicted equilibria (asymptotes) of total radioactivity in the fish were derived by fitting the observed data to a Gompertz curve (Croxton and Cowden 1955). The half-life of DEHP

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Di-2-ethylhexyl phthalate (DEHP) is the most widely used plasticizer in polyvinyl chloride plastics (U.S. Tariff Commission 1972), and the finished plastic product may contain up to 60% of DEHP by weight (Nematollahi et al. 1967). The ubiquity of DEHP as a contaminant in various constituents of aquatic ecosystems was described by Mayer et al. (1972). DEHP has also been reported to occur naturally (Mathur 1974). The toxicity of DEHP and its effects on reproduction in aquatic organisms were described by Mayer and Sanders (1973) and Sanders et al. (1973), and DEHP degradation in fishes was evaluated by Metcalf et al. (1973) and Stalling et al. (1973). DEHP has been found degradable in soil (Mathur 1974), sediments (Johnson and Lulves 1975), sludge (Saeger and Tucker 1973), and by an almost ubiquitous bacterial saprophyte (Mathur and Rouatt 1975). Because little is known of the residue dynamics of DEHP in fishes, the present study was undertaken to elucidate the uptake, storage, degradation, and elimination of the chemical in the fathead minnow (Pimephales promelas).

Materials and methods - The study was conducted in a flow-through diluter system modified from Mount and Brungs (1967) and consisted of seven concentrations of DEHP (1.9, 2.5, 4.6, 8.1, 14, 30, and 62 µg/liter) and a control. Exposure containers were glass aquaria, 30.5 cm deep x 61 cm long x 30.5 cm wide, containing 42 liters of well water (pH 7.4, total hardness 270 mg/liter as $CaCO_3$) maintained at 25±0.5 C. Stock solutions of ^{14}C-carbonyl-labeled DEHP (1.64 mCi/mmole) mixed

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ABSTRACT

Mayer, F.L. 1976. Residue Dynamics of Di-2-ethylhexyl Phthalate in Fathead Minnows (Pimephales promelas). J. Fish. Res. Board Can. 33:000-000.

Fathead minnows (Pimephales promelas) were continuously exposed to 1.9 to 62 µg/liter di-2-ethylhexyl phthalate (DEHP) for 56 days. The accumulation factors ranged downward from 886 to 155 as the exposure concentrations increased. The major metabolite of DEHP was the monoester, 2-ethylhexyl phthalate. After being placed in uncontaminated water, the fish eliminated half of the DEHP in 12.2 days.

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Residue Dynamics of Di-2-ethylhexyl Phthalate in Fathead Minnows (Pimephales promelas)

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