Fate of ^{14}C-benzene in Eggs and Larvae of Pacific Herring (*Clupea harengus pallasi*)

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Eggs and larvae of Pacific herring were exposed to low concentrations of ^{14}C-labeled benzene, a soluble, aromatic component of crude oil. All life stages accumulated benzene from water in direct proportion to the initial exposure concentrations, reaching equilibrium within 6–12 h. Feeding larvae exposed to benzene through water and live food accumulated benzene initially from the water, then secondarily from the food. The maximum amount of benzene accumulated in tissues was inversely related to age. Eggs accumulated up to 10.9 times the initial concentration, yolk-sac larvae up to 6.9 times, and feeding larvae to 3.9 times. Reasons for these differences in total accumulation and uptake rate are discussed. Results provide evidence for food web magnification of petroleum-based hydrocarbons.

Key words: Pacific herring, *Clupea harengus pallasi*, eggs and larvae, ^{14}C-benzene, uptake, depuration, food chain, petroleum-based hydrocarbons

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Des oeufs et des larves de hareng du Pacifique furent exposés à de faibles concentrations de benzène, un constituant aromatique soluble du pétrole brut, marqué au ^{14}C. Tous les stades biologiques accumulent du benzène en provenance de l'eau en proportion directe des concentrations initiales d'exposition, jusqu'à ce qu'un équilibre s'établisse en dedans de 6 à 12 h. Des larves capables de se nourrir, exposées à du benzène à la fois dans l'eau et dans leur nourriture vivante, accumulent le benzène d'abord de l'eau, ensuite de la nourriture. La quantité maximale de benzène accumulée dans les tissus est en relation inverse de l'âge. Les oeufs accumulent jusqu'à 10.9 fois la concentration initiale, les larves vésiculées jusqu'à 6.9 fois et les larves qui se nourrissent jusqu'à 3.9 fois. Nous analysons les raisons de ces différences dans l'accumulation totale et le taux d'assimilation. Ces résultats sont une preuve de l'accroissement, dans le réseau alimentaire, des hydrocarbures du pétrole.

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NUMEROUS researchers have shown that marine organisms representing different species and trophic levels take up and release petroleum hydrocarbons (Lee et al. 1972; Teal and Stegeman 1973; Vaughan 1973; Anderson et al. 1974; Fossato 1975; Lee 1975; Corner et al. 1976; Korn et al. 1976). In radiometric studies marine organisms more rapidly metabolized *n*-alkanes than aromatics (Lee 1975).

Studies of the relatively more toxic aromatic fractions have concentrated on the higher aromatics and only lately have investigators examined benzene, a relatively abundant (up to 6.75 mg/L in the water-soluble fraction) and soluble aromatic (1993 μL/L) component of crude oil (Anderson et al. 1974; Benville and Korn 1974). Benzene at concentrations of 20–45 μL/L is acutely toxic and at 5–10 μL/L is chronically toxic to Pacific herring (*Clupea harengus pallasi*) eggs and larvae (Struhsaker et al. 1974) and at 2–11 μL/L is acutely toxic to various juvenile and adult fishes (Meyerhoff 1975; Benville and Korn 1977). Korn et al. (1976, 1977) and Roubal et al. (1977) found that in juvenile and adult fishes aqueous benzene rapidly entered the fish and was metabolized in the liver. In nearly all studies

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TABLE 1. Summary of experimental variables for ^{14}C-benzene uptake and depuration tests with Pacific herring embryos and larvae. All tissues were sampled at 6, 12, 24, 48, and 72 h except marked (*) treatments, which ended at 48 h.

Life stage	Type of exposure	Initial mean benzene concn (μL/L)	Specific activity (cpm/nL)	Total wet wt (mg) mean	±SD	Estimated no. of individuals per sample
Egg—embryo	Benzene-treated water	0.063	20.00	158.9	±21.3	81
		0.560	2.00	155.3	±16.0	79
Yolk-sac larva	Benzene-treated water	0.036	13.00	147.2	±56.2	118
		0.460	0.90	127.9	±46.6	102
Postyolk-sac larva	Benzene-treated water	0.144	2.20	154.5	±35.5	144
		1.200*	5.00	135.1	±36.1	126
		2.100	0.14	170.4	±40.4	158
	Benzene-treated food and clean water	1.200*	5.00	117.5	±25.6	109
	Benzene-treated water and clean food	1.440*	2.20	149.7	±70.1	139
		2.100	0.14	148.8	±65.7	138
	Benzene-treated food and water	1.200*	5.00	129.2	±43.1	120

juvenile and/or adult life stages were used, and they were only exposed to hydrocarbons in the water.

The object of this study was to determine the fate of benzene in the eggs, yolk-sac larvae, and feeding larvae of Pacific herring. We wished to compare the uptake of benzene from the water with that from food and determine whether biomagnification occurred, as has been demonstrated with pesticides (Hickey and Keith 1965; Woodwell et al. 1967; Macek and Korn 1970; Reinert 1972).

Materials and Methods

TEST ANIMALS

For experiments with eggs, Pacific herring (*Clupea harengus pallasi*) eggs were artificially fertilized with gametes of freshly caught, sexually mature adults. A single layer of eggs was spread over 10 × 24 cm glass plates and placed in green polyethylene-plastic rectangular pans filled with 8 L of filtered (pore size 2 μm) seawater. In the experiments with larvae, naturally fertilized eggs were gathered in San Francisco Bay and incubated in similar pans. After hatching, larvae were transferred to new pans filled with freshly filtered seawater.

Postyolk-sac feeding larvae (9.4 mm mean standard length) were fed marine rotifers (*Brachionus plicatilis*) which were cultured according to the techniques of Theilacker and McMaster (1971), except that the small green flagellate (*Nephroselmis* sp.) was used as rotifer food. Rotifer concentrations were maintained at 14–20 rotifers/mL as determined with a Model ZBI Coulter counter.

EXPERIMENTAL CONDITIONS

Experiments were conducted in the previously described 8-L pans, under static conditions. Freshly filtered seawater was added at the beginning of each experiment, and temperature, dissolved oxygen, and salinity were measured daily. To stabilize water conditions pans were held in water baths under constant light (about 1900 lx). Dissolved oxygen values were at or near saturation, salinities were constant at 23.0‰, and temperature ranged from 12.2 to 13.5°C throughout the experiments.

EXPOSURE PROCEDURES

Animals were given a single dose of benzene just after fertilization, just after hatching (yolk-sac larvae), and at completion of yolk absorption (feeding larvae). They were therefore exposed to continuously declining concentrations of benzene, which from previous experiments has been shown to decline exponentially to ~25–30% of the initial concentration within 24 h (Struhsaker et al. 1974). Aqueous benzene was introduced directly into the water in the form of benzene-saturated seawater (1 mL benzene to 250 mL seawater vigorously shaken and settled for 1 h). Ring-labeled ^{14}C-benzene of the desired specific activity (Table 1) was added to this stock solution and the appropriate amount of benzene added to the test containers. An attempt was made to introduce benzene in two concentrations of 1.00 and 0.10 μL/L. Due to its volatility and the difficulty in dissolving benzene, the actual in situ concentrations of benzene ranged from 0.010 to 2.100 μL/L as determined by gas chromatography (Table 1). In the feeding larval experiment ^{14}C-labeled benzene was introduced in three ways: (a) in contaminated water only, without food, (b) in benzene-contaminated rotifers only, and (c) in benzene-contaminated water and benzene-contaminated rotifers.

We contaminated the rotifers by introducing ^{14}C-labeled benzene to the rotifer rearing container at ~0.1 and 1.0 μL/L concentrations. The rotifers were allowed to accumulate benzene for 2 d. They were then removed, rinsed with fresh seawater, and fed to the larvae.

WATER AND TISSUE ANALYSES

Two water and tissue samples were taken at 6-, 12-, 24-, 48-, and 72-h intervals. The 1-mL water samples were added to 10 mL Instagel scintillator and counted in a Packard Tri Carb scintillation spectrometer. For tissue samples live larvae were rinsed with distilled water, weighed (Table 1), placed in a vial with solubilizing solution (1 mL/100 mg tissue Packard Soluene-100) for 48 h and combined with 10 mL of scintillator (Packard Dimilume). To avoid rapid drying of the samples and the potential loss of benzene we

TABLE 2. Decreasing concentrations (μL/L) over 48 h of ^{14}C-labeled benzene in the water of experiments with Pacific herring eggs and larvae.

Expt	Life stage	Hours					% volatilized at 24 h
		0	6	12	24	48	
1	Embryo	0.063	0.007	0.007	0.003	—[a]	60
1	Embryo	0.560	0.035	0.029	0.023	—	77
2	Yolk-sac larva	0.036	0.023	0.020	0.018	—	50
2	Yolk-sac larva	0.460	0.356	0.329	0.205	—	55
3	Postyolk-sac larva	0.144	0.088	—	0.056	—	61
3	Postyolk-sac larva	2.100	1.320	—	0.723	—	68
4	Postyolk-sac larva	1.200	0.800	0.580	0.360	—	60

[a]No detectable concentration.

did not count the numbers of individuals in each sample. Rather, we estimated their numbers (Table 1) by using Ehrlich's (1972) determinations of water content and our own dry weight measurements. Method efficiency was 61%, predetermined with spiked samples of known counts per minute (cpm). Water and tissue samples with emissions below 40 cpm were considered below the detectable limits of our system.

Radiometric analysis measures ^{14}C and cannot determine if the label remains on an intact benzene ring or on a metabolite of benzene. Since we did not analyze for the presence of benzene metabolites, all results and discussion on accumulation and depuration must be considered to represent benzene and/or benzene metabolites.

Results and Discussion

Aqueous benzene concentrations in our test containers decreased exponentially in the first 24 h after introduction (Table 2) as was found in previous larval fish experiments (Struhsaker et al. 1974); benzene was absent after 48 h.

Embryos and yolk-sac larvae responded to single exposures of aqueous benzene in almost identical fashion. Both rapidly accumulated benzene to maximum levels within 6–12 h after exposure (Table 3). Embryos then lost benzene at an exponential rate, declining to levels just above the initial exposure concentrations. Yolk-sac larvae began depurating between 24 and 48 h but differed from embryos by exhibiting an increase in ^{14}C-benzene between 48 and 72 h. How yolk-sac larvae accumulated benzene after a period of depuration is difficult to explain, since free benzene was not detectable in the water. However, fish larvae are known to increase their tissue water content when starved (Ehrlich 1974). Until feeding is well established some tissue resorption takes place, a condition which, if continued, would lead to starvation and eventual death. Benzene may enter larvae via infused water.

Like embryos and yolk-sac larvae, nonfeeding postyolk-sac larvae exposed to three aqueous concentrations (0.144, 1.200, and 2.100 μL/L) quickly absorbed benzene, attaining maximum accumulations within 6–12 h (Table 3). Subsequent depuration followed with leveling off at or near the original exposure concentrations.

Postyolk-sac larvae feeding on contaminated rotifers or rotifers in contaminated water demonstrated a bimodal uptake response to benzene exposure. When benzene in situ concentrations of 0.140 and 2.100 μL/g were added to water containing larvae actively feeding on clean rotifers, larvae rapidly accumulated benzene during the first 6 h (Table 3). This initial accumulation was similar to the previously described response of unfed larvae and presumably represents uptake of aqueous benzene. Depuration from larval tissues between 6 and 24 h was slow or nonexistent, and after this period uptake was again exhibited. Maximum concentrations of 0.49 and 8.160 μL/g were reached at 48 and 72 h for the low and high exposure concentrations, respectively. Larvae apparently ingested rotifers during this latter period which had become contaminated from the aqueous benzene. In a separate treatment larvae exposed to both contaminated water and precontaminated rotifers responded the same as larvae exposed to benzene-contaminated water only and clean rotifers (the 0.140 and 2.100 μL/L of Table 3). The initial benzene accumulation in the doubly exposed larvae reached 2.47 and the second buildup reached 3.98 μL/L. In the final treatment, larvae that fed on only rotifers previously contaminated with 1.200 μL/L benzene experienced no initial rapid accumulation; rather a steady rise in contamination to 0.310 μL/L was exhibited.

The secondary accumulation of benzene by ingestion of rotifers appeared to be a lower but potentially more accumulative process. The principal reason for this is that *B. plicatilis* appears incapable of either discharging or metabolizing benzene for time periods far exceeding the developmental period when herring larvae would depend on them for food (Echeverria unpublished data). In experiments conducted at our laboratory we found rotifers accumulated and retained benzene and/or its metabolites 10^3–10^4 times the original concentration for periods up to 8 d. Larvae fed on rotifers that in turn were accumulating higher concentrations of ^{14}C hydrocarbons. The longer time period needed for rotifers to

TABLE 3. Means ±SE of benzene concentrations (nL/g) in exposed Pacific herring eggs and larvae.

Life stage	Initial exposure concn (μL/L)	Hours				
		6	12	24	48	72
Embryo	0.063	0.58±0.04	0.61±0.01	0.32±0.02	0.18±0.01	0.10±0.00
	0.560	6.10±0.30	6.14±0.19	3.45±0.14	1.26±0.02	0.91±0.00[a]
Yolk-sac larva	0.036	0.25±0.01	0.13±0.02	0.09±0.01	0.10±0.00	0.39±0.01
	0.460	2.40±0.15	3.12±0.19	1.81±0.10	0.67±0.02	0.89±0.02
Unfed larva	0.144	0.22±0.02	0.23±0.02	0.17±0.01	0.18±0.03	0.17±0.00[a]
	1.200	3.21±0.00[a]	3.10±0.09	1.44±0.09	1.24±0.21	—
	2.100	4.09±0.09	4.06±0.09	2.84±0.19	2.78±0.02	2.74±0.05
Fed larva	0.140	0.23±0.01	0.25±0.01	0.26±0.00	0.49±0.07	—
	1.200	0.04±0.01	0.10±0.05	0.22±0.07	0.31±0.04	—
	1.200	2.47±0.04	2.28±0.04	1.78±0.04	3.98±0.00[a]	—
	2.100	4.99±0.04	4.23±0.03	3.27±0.05	6.22±0.12	8.16±0.15

[a]Only one sample used for calculation.

take up and concentrate these hydrocarbons explains the secondary or delayed response in herring uptake. The presence of aqueous benzene seems to affect the amount of benzene taken up by rotifers and hence by larvae. When contaminated rotifers alone were present, larvae accumulated only 0.3 μL/L of the original concentration. Further sampling beyond 48 h is needed to determine whether larvae would continue to accumulate benzene. In a simple comparison between results (Table 3), we calculated the relative contribution of benzene derived from the water versus from the food. In the first rapid uptake an average of 91% of tissue benzene was obtained from the water. The second peak at 48 h was an average of 38% from the water and 62% from rotifers. Presumably larvae would proportionally take up more benzene from rotifers in time as waterborne benzene concentrations declined and rotifers accumulated more.

The maximum amount of benzene accumulated in tissues differed among early life stages. The younger the stage, the higher the concentration factor. Eggs absorbed benzene 9.7 and 10.9 times the concentrations added to the water, and yolk-sac larvae accumulated 6.9 and 6.8 times the initial concentration. After yolk absorption, larvae accumulated less benzene than either eggs or yolk-sac larvae. Factors ranged from 0.3 for larvae feeding on precontaminated rotifers to 3.9 times the initial exposure concentration in larvae feeding on rotifers in benzene-contaminated water. This highest accumulation of benzene occurred 72 h after exposure (Table 3) when larvae were feeding on rotifers and there was no indication of a decrease in accumulation. The uptake patterns seen in the accumulations of other postyolk-sac larvae fed rotifers (Table 3) also suggest maximum levels had not been reached. No samples were taken after 72 h.

All stages accumulated benzene rapidly from the water, but those with yolk exhibited higher tissue magnification factors than larvae past the stage of yolk absorption. Higher concentration factors in these yolk-containing stages are most likely caused by two factors: the high lipid content of yolk-bearing stages and the lack of specialized tissues. Korn et al. (1976) found benzene accumulated predominantly in tissues with high lipid content in teleosts. Lipophilic benzene probably accumulated in the lipid-rich yolk of the herring eggs and yolk-sac larvae. The content of lipids in conspecific Atlantic herring (*Clupea harengus harengus*) eggs has been determined by Ehrlich (1972) to be 18.12% of egg dry weight. In related experiments we measured oxygen consumption which showed herring larvae had much higher metabolic rates than eggs. This would mean utilization of lipid-rich yolk and excretion rates would be less in embryos than in active larvae, thereby affecting the turnover rate of benzene in the two life stages.

It is commonly believed the liver is a major site of petroleum hydrocarbon metabolism. Livers and gallbladders of adult fishes have been found to accumulate the highest concentrations of benzene over all tissues tested (Korn et al. 1976; Roubal et al. 1977). The metabolism and/or depuration of benzene is likely slower in the less-developed life stages lacking these functional organs.

Until recently no evidence existed for food-web magnification of petroleum-based hydrocarbons (Lu and Metcalf 1975; National Academy of Sciences 1975; Koons et al. 1976). Corner et al. (1976), however, found that *Calanus helgolandicus* responded to naphthalene exposure in much the same manner as did herring larvae. Hydrocarbons accumulated through the diet were quantitatively more important than those taken up directly from solution. Accumulation and depuration of hydrocarbons were much slower when obtained from contaminated food. Results of our study clearly demonstrated that fish larvae feeding on zooplankters that cannot metabolize or discharge petro-

leum hydrocarbons in short time periods do accumulate hydrocarbons to amounts in excess of the exposure concentrations. Further, zooplankters of this type or copepods with high hydrocarbon contents provide a pathway of entry for petroleum hydrocarbons that may be more significant than through the water.

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