FATE OF ^{14}C–BENZENE (AN AROMATIC HYDROCARBON OF CRUDE OIL) IN A SIMPLE FOOD CHAIN OF ROTIFERS AND PACIFIC HERRING LARVAE

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Abstract. Increased ocean spillage of petroleum hydrocarbons has led scientists to study the uptake, accumulation and depuration of specific hydrocarbons in invertebrates and fishes representing different species and trophic levels.

This paper presents results of experiments in which newly feeding post-yolk-sac larvae of Pacific herring were exposed to ^{14}C-labeled benzene through water and live food. Larvae had a bimodal response to benzene exposure. Benzene and/or its metabolites were seen to accumulate rapidly (within 6 to 12 hours) from the water to levels approximately 2 times the exposure concentration. When contaminated marine rotifers were present, larvae exhibited a secondary accumulation starting at 24 hours after exposure. Higher average concentration factors of 3.2 were found after 48 hours.

These studies showed petroleum hydrocarbons are taken up and accumulated from both water and food, and predators concentrate these hydrocarbons or their metabolites to levels in excess of initial exposure concentrations.

INTRODUCTION

A recent report estimated the worldwide input of oil to the oceans from all sources to be 6 million metric tons per year (National Academy of Sciences 1975). To assess the impact of these spills on the environment scientists have studied the effects of exposure to petroleum hydrocarbons in field surveys, acute and chronic bioassays, and uptake and accumulation studies. In the latter area of research it has been demonstrated that most invertebrates and fishes representing different species and trophic levels rapidly accumulate water-borne petroleum hydrocarbons often to levels 200-300 times the original exposure concentration (Lee et al. 1972(a); Neff and Anderson 1975; Lee et al. 1972(b); Roubal et al. 1977). It was also shown that once exposure ceased, slower, but still rapid discharge of the hydrocarbons occurred. While these animals demonstrated effects due to exposure of oil in water, another major important pathway is through food. Copepods and

barnacle nauplii have been found to ingest oil directly (Parker et al. 1971; Conover 1971). Adult plaice readily ate shrimp contaminated with oil (Blackman and Mackie 1974).

These previous research efforts did not test for biomagnification of hydrocarbons between trophic levels. The potential for magnification is especially great between lower phylogenetic levels where the tendency is to be unable to metabolize hydrocarbons.

In this research we created a simple food chain using newly feeding Pacific herring (Clupea harengus pallasi) larvae and the marine rotifer Brachionus plicatilis. Herring larvae at the end of their yolk absorption must successfully change from an internal energy source (yolk) to an external one (food) in order to survive. This time is often referred to as the "critical period" (May 1974). We have successfully used rotifers as an initial food in the rearing of herring larvae, and it has been used with other fish species as well (Theilacker and McMaster 1971). It is an abundant food organism in both fresh and saline waters, and we feel it well represents a typical food item for beginning larval fish feeders.

Benzene is a commonly used aromatic hydrocarbon. It is a major component of the water-soluble fraction of crude oil (Anderson et al. 1974) and is relatively soluble in seawater (Benville and Korn 1974).

Its acute toxicity (96-hr LC_{50}) to adult striped bass was determined to be 2.0 to 11.0 µl/liter (Benville and Korn, in press); to juvenile striped bass, 10.9 µl/liter (Meyerhoff 1975); and both acute and chronic effects of benzene were noted in eggs and larvae of herring and northern anchovy exposed to an initial concentration range of 5 to 50 µl/liter (Struhsaker et al. 1974).

This paper presents results of a series of experiments designed to determine the degree to which Pacific herring larvae take up, accumulate, and depurate ^{14}C-labeled benzene presented in the water and food.

We wish to thank Sid Korn of the NMFS Auke Bay, Alaska, Laboratory for assistance in the radiometric analyses.

METHODS AND MATERIALS

Experimental Animals

The rotifer Brachionus plicatilis was cultured in 80-liter glass aquaria under constant aeration, light (Grow-Lux and Day Light fluorescent lights @ 20 fc), salinity (20 °/oo ± 2 °/oo) and temperature (22° ± 2°C). They were daily fed a small (2µ) green flagellate Nephroselmis sp. to ensure a healthy population. Rotifers were given to the larvae in densities of 15 to 20 rotifers/ml, determined with the use of a Coulter Counter, model ZBI.

Herring larvae were obtained from naturally fertilized eggs spawned on algae in San Francisco Bay. Eggs were incubated for 9 days in polyethylene plastic pans containing 8 liters of filtered seawater (pore size 5µ) with an antibiotic (erythromycin gluceptate) treatment of 5 ppm. Water in the pans was changed prior to hatching and every two days afterward until the beginning of the exposure periods. Five days after hatching the yolk was totally consumed and feeding and exposures were begun. Daily measurements of water quality in the pans showed dissolved oxygen values were at or near saturation, salinities were constant at 23 °/oo and temperatures ranged 12.2 to 13.5°C.

Fig. 1. Concentrations of ^{14}C-labeled benzene and/or its metabolites in unfed post-yolksac larvae of Pacific herring.

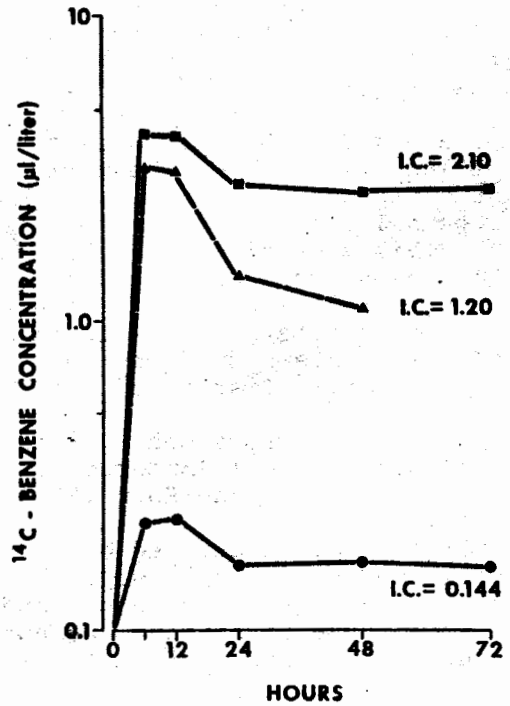
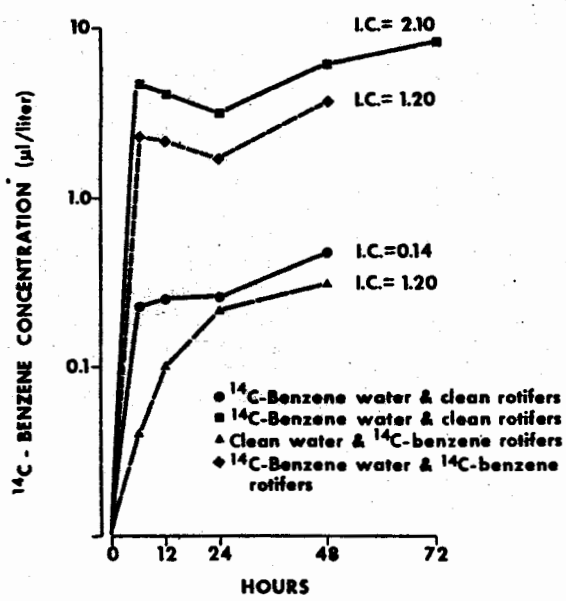


Fig. 2. Concentrations of ^{14}C-labeled benzene and/or its metabolites in Pacific herring post-yolksac larvae exposed to benzene in different combinations of water and food.



Exposures

All benzene exposures were single doses and static. All tests were conducted in the previously mentioned plastic pans. To contaminate the rotifers we concentrated them on a 53μ Vitex nylon net, rinsed with clean, filtered seawater and transferred them to the test containers. Benzene was introduced in benzene saturated seawater (1 ml benzene to 250 ml seawater vigorously shaken and settled for 1 hr). Rotifers were allowed to concentrate the benzene for 48 hours, then they were filtered, rinsed and fed to the larvae. Besides being exposed to benzene through the food, larvae were also challenged with benzene in the water. One treatment contained food in the water so that rotifers and larvae were exposed simultaneously and another treatment had unfed larvae in it.

Benzene concentrations were sublethal, calculated to give initial concentrations of either 0.1 or 1.0 μl/liter. The appropriate amount of ring-labeled ^{14}C-benzene was added to the stock solution to give the desired specific activity (Table 1). Because of benzene's volatility, true water concentrations were measured by gas chromatography (Table 1) and ranged 0.144 to 2.100 μl/l.

Sample Analyses

Two water and two tissue samples were taken at each 6, 12, 24, 48, and 72-hour interval. The one ml water samples were added to 10 mls of instagel scintillator and placed in the scintillation spectrometer. For tissue samples live larvae were rinsed with distilled water, weighed (Table 1), placed in a vial with digestor solution (1 ml/100 mg tissue Packard Soluene-100) for 48 hours, and combined with 10-ml of scintillator (Packard Dimilume). Radioactivity was measured on a Packard Model 2008 tri-Carb liquid scintillation spectrometer system. Approximate method efficiency was 61%, predetermined by spiked samples of known concentrations. Water and tissue samples with emissions below 40 counts per minute were considered below the detectable limits of our system.

Radiometric analysis measures radioactive ^{14}C. It does not necessarily determine the presence of benzene. ^{14}C could also be present in the form of any number of metabolic compounds derived from benzene. We did not analyze for the presence of benzene metabolites. Therefore all results and discussion on ^{14}C uptake, accumulation and depuration must be considered to represent benzene and/or benzene metabolites.

RESULTS

As in previous experiments with identical containers and dosing procedures, benzene concentrations declined exponentially ($\hat{Y}=a\ e^{-bx}$) in the first 24 hours (Struhsaker *et al.* 1974). An average 37% of the initial concentrations remained in the pans 24 hours after dosing. By 48 hours no aqueous ^{14}C was detectable.

Non-feeding post yolksac larvae exposed to aqueous concentrations of 0.144, 1.200 and 2.100 μl/liter benzene quickly took up benzene reaching maximum accumulations of 0.23, 3.21 and 4.09 μl/liter between 6 and 12 hours (Figure 1). Depuration followed with a gradual leveling off at or near exposure concentrations.

When feeding larvae and uncontaminated rotifers were challenged with waterborne benzene, the larvae's first response was to accumulate benzene as did the nonfeeding larvae. They rapidly took up benzene within 6 to 12 hours to concentrations of 0.25 and 4.99 μl/liter (Figure 2) from exposure concentrations of 0.14 and 2.10 μl/liter. This primary accumulation was followed by either depuration or leveling off for the succeeding 12 hours. Then a secondary accumulation was seen beginning at 24 hours and proceeded

to higher maximums of 0.49 and 8.16 µl/liter at 48 and 72 hours respectively.

Larvae which were given previously contaminated rotifers and aqueous benzene (1.20 µl/liter) simultaneously, accumulated benzene the same as the previously treated larvae (Figure 2). Their initial uptake was 2.47 and their second 3.98 µl/liter.

Larvae feeding on previously contaminated (1.20 µl/liter) rotifers only, had no initial accumulation, rather a continuous increase to 0.31 µl/liter within 48 hours.

In the initial uptake response of larvae exposed to aqueous benzene, concentration factors averaged 2.1 (unfed larvae) and 2.0 (fed larvae). The secondary accumulations were higher, having an average 3.2 at 48 hours. Another tissue sample of the 2.10 µl/liter exposure at 72 hours indicated a larger factor of 3.9 suggesting accumulation was continuing.

The larvae exposed to benzene in previously contaminated rotifers alone did not accumulate as much (only 0.3 the initial concentration) as larvae given aqueous benzene. As before, Figure 2 illustrates how concentrations seem to have been rising, suggesting maximum accumulations had not yet been reached.

DISCUSSION

"There is no evidence for food web magnification of petroleum hydrocarbons in marine organisms" (National Academy of Sciences, 1975).

"Because marine organisms lose hydrocarbons they may have accumulated, contamination cannot become concentrated by transfer to other trophic levels ..." (Koons et al. 1976).

Results presented in this paper provide evidence that petroleum hydrocarbons are taken up and accumulated from both the water and food and predators do concentrate these hydrocarbons or their metabolites to levels in excess of the initial exposure concentration. Post yolksac herring larvae appeared more vulnerable to hydrocarbon uptake when exposed directly from the water. However, as in juvenile and adult fishes, both uptake and depuration were rapid. Feeding larvae on the other hand accumulated benzene from live food in a delayed response. A significant factor in this secondary uptake was that rotifers are incapable of either discharging or metabolizing benzene and/or its metabolites (Echeverria, manuscript in preparation). In similar benzene exposure concentrations of 1.0 and 0.1 µl/liter, rotifers accumulated ^{14}C-labeled benzene compounds 10^3-10^4 times the water concentration. In chronic exposures they retained high levels (10^2-10^3X) for up to 6 days after exposures ceased. It is possible that should a food organism be able to release petroleum hydrocarbons rapidly, concentration factors we found in herring larvae would not occur, assuming a single exposure situation as was used in these experiments.

Although feeding herring larvae accumulated benzene and its metabolites up to 3.9 times the initial exposure, the concentration factors of larvae were much lower than those found in adult northern anchovy and striped bass (Korn et al. 1976). These adult fishes accumulated 53.4 to 8,450 times the initial water concentration in the gall bladder. Benzene and/or metabolites concentrated in tissues with high lipid content or in major metabolic sites.

The difference between larval and adult uptake levels is most likely due to the length of exposure (one single dose in our experiments vs. 48-hour exposure (one single dose in our experiments vs. 48-hour exposures for adults) and the size and organ development of larvae vs. adults. Metabolism of petroleum hydrocarbons in adults apparently takes place in the liver.

Metabolites concentrate in the gall bladder and ultimately are passed through the intestine. Access of aqueous or food-borne hydrocarbons to larval tissues is more direct and specialized organ size and functions are in a state of beginning development when compared to adults. The reverse is also true. Release of hydrocarbons from larval tissues can be rapid after exposure ceases, mainly because of the large volume to weight ratio and the thin tissue layers. If larvae have rapid depuration capability, it is probable the feeding larvae in our experiments had not reached their maximum accumulation levels. This is supported by the greater concentration factor of larvae sampled at 72 hours compared to 48 hours. Equilibrium levels need to be determined which would require sampling beyond 72 hours after exposure.

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Table 1. Summary of experimental variables for ^{14}C-benzene uptake and discharge tests with Pacific herring embryos and larvae.

Type of Exposure	Initial Mean Benzene Concentration (µl/liter)	Specific Activity (cpm/nl)	Time of Tissue Sampling (hours)	Total Wet Weight (mg) Mean	S.D.
Water (not fed)	0.144	2.2	Same	154.5	35.5
	1.200	5	6,12,24,48	135.1	36.1
	2.100	0.135	6,12,24,48 72	170.4	40.4
Food and Clean water	1.200	5	6,12,24,48	117.5	25.6
Water and Clean food	1.440	2.2	6,12,24,48	149.7	70.1
	2.100	0.135	6,12,24,48 72	148.8	65.7
Food and Water	1.20	5	6,12,24,48	129.2	43.1