Accumulation of ^{14}C Labeled Benzene and Related Compounds in the Rotifer *Brachionus plicatilis* from Seawater

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ECHEVERRIA, T. 1980. Accumulation of ^{14}C labeled benzene and related compounds in the rotifer *Brachionus plicatilis* from seawater. Can. J. Fish. Aquat. Sci. 37: 738–741.

Rotifers were exposed to (1) a single dose of ^{14}C labeled benzene on day 1 with initial concentrations of benzene at 0.1 and 1.0 μL/L declining over time and (2) a chronic exposure of 0.1 and 1.0 μL/L daily for 9 d. Both conditions resulted in rotifers accumulating 1000 to 10 000 times the ^{14}C activity detected in the seawater. These concentrations were maintained in the rotifers for 8 and 11 d, respectively, and remained high even after exposure was terminated. Analysis of ^{14}C activity from ^{14}C labeled benzene detected in the water revealed its source to be phenolic compounds, compounds with a potentially higher toxicity than benzene. The water maintained a low level of ^{14}C compounds throughout the experiments. Rotifers accumulated high quantities and retained most of the acquired ^{14}C compounds after 2 d in clean water.

Key words: benzene, rotifer, zooplankton, biomagnification

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Des rotifères furent soumis à (1) une exposition à une seule dose de benzène marqué au ^{14}C au jour 1, de concentrations initiales de benzène de 0,1 et 1,0 μL/L, diminuant avec le temps, et (2) une exposition chronique à 0,1 et 1,0 μL/L quotidiennement pendant 9 jours. Dans les deux situations, il en est résulté chez les rotifères une accumulation de 1 000 à 10 000 fois l'activité du ^{14}C décelée dans l'eau de mer. Ces concentrations se maintiennent chez les rotifères pendant 8 et 11 jours, respectivement, et demeurent à un niveau élevé même une fois l'exposition terminée. L'analyse de l'activité du ^{14}C dans le benzène marqué au ^{14}C décelée dans l'eau montre qu'elle a comme source des composés phénoliques, composés d'une toxicité potentiellement supérieure à celle du benzène. Durant toutes les expériences, l'eau conserve un bas niveau de composés de ^{14}C. Les rotifères accumulent de fortes quantités et conservent la plus grande partie des composée de ^{14}C acquis, après 2 jours en eau propre.

Received January 17, 1978
Accepted January 8, 1979

Reçu le 17 janvier 1978
Accepté le 8 janvier 1979

METABOLISM of petroleum hydrocarbons by animals requires certain NADPH-dependent enzymes (Sims and Grover 1974). Many marine fish and invertebrates are able to metabolize hydrocarbons (Varanasi and Malins 1977). Some marine phytoplankton and invertebrates are unable to metabolize specific hydrocarbons because they do not possess the necessary enzymes. The ability of clams (Neff and Anderson 1975), mussels (Clark and Finley 1975; Lee et al. 1972), barnacles (Morris 1973), oysters (Stegeman and Teal 1973), and jellyfish (Lee 1975) to accumulate oil components, but not to metabolize them, stimulated my interest in biomagnification through the food chain.

The cosmopolitan euryhaline rotifer *Brachionus plicatilis* is easily reared in the laboratory and has proven to be adequate as larval fish food (Theilacker and McMaster 1971). It is now used in the Tiburon Laboratory to rear larval fishes (Struhsaker et al. 1974).

Benzene, a monocyclic aromatic hydrocarbon of the water-soluble fraction of crude oil, has a low toxicity (Hawley 1971) and is soluble in seawater at 1400 μL/L (Benville and Korn 1977). Benzene occurs chronically in the open ocean and in estuaries at about 0.001 to 0.1 μL/L (Lee and Ryan 1976).

To determine the fate of a single component of crude oil in a prey species, I studied the uptake and depuration of benzene in *B. plicatilis* exposed to experimental concentrations of benzene that approximated measured environmental levels.

Methods — A culture of *B. plicatilis* was maintained in an 80-L glass aquarium under constant aeration, illumination (Grolux[1] and fluorescent lights at 215 lx), salinity (20‰ ± 2‰), and temperature (22 ± 2°C). They were fed a 2-μm-long green flagellate (*Nephroselmis* sp.: 10^4 cells/mL) reared under identical conditions, except for a higher light intensity (1615 lx). The rotifers were fed daily for 1 wk before experiments to ensure a healthy population, but not during the experiment.

Double cellulose-filtered (pore size 5 μm) and 8-L single charcoal-filtered seawater was placed in green, 10-L poly-

Printed in Canada (J5099)
Imprimé au Canada (J5099)

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service (NOAA).

ethylene pans. The static pans were kept in a water bath at constant light (24 h: 108 lx), temperature (14–16°C), and salinity (23‰). Rotifers were concentrated on a 53-μm Nitex nylon net, rinsed with clean, filtered seawater, and transferred to the experiment pans. Rotifer populations were monitored daily using a model ZBI Coulter counter. Rotifer dry weights were obtained by rinsing with distilled water, drying at 20°C for 24 h, and weighing on a Cahn model 4400 electro-balance (±0.1 μg). The average weight for a rotifer was 0.35 μg. [^{14}C]benzene solution was prepared by combining a benzene stock solution with ^{14}C labeled benzene. The saturated benzene stock solution was prepared by vigorously shaking 99.9% benzene into 1 L of filtered seawater. The concentration was measured on a Tracor MT220 gas chromatograph (GC) (Benville and Korn 1974). An appropriate amount of stock solution was combined with ^{14}C labeled benzene to give the desired specific activity, and then stirred into pans for final concentrations of 0.1 or 1.0 μL/L. [^{14}C]benzene solution activities were monitored by sampling 1 mL from the center of each pan, mixing it with 10 mL Instagel® scintillator, and counting it on a Packard liquid scintillation spectrometer. The activity of experimental seawater was similarly measured daily, before and after the addition of fresh [^{14}C]benzene solution. Initial measurements were used to calculate how much, if any, new [^{14}C]benzene solution is needed to maintain specific activities of 10 and 100 in expt. 1, and 12 and 122 in expt. 2. Water samples were measured on a gas chromatograph (GC) on days 1, 3, and 5 to determine actual amounts of benzene (Fig. 1).

The activity in rotifers was counted by concentrating up to 500 mL of rotifers, or ~0.02 mg on a 53-μm net, washed into a beaker with 10 mL of water, and transferred to a Pasteur capillary pipet plugged at the base with glass wool. Packard-100® solution digestor was added (0.5 mL), and the pipet sealed for 25 h at 20°C. The digest was transferred to a scintillation vial with 10-mL Dimulene® scintillator by using air pressure and then flushing the pipet twice with the scintillator. Samples were counted and corrected for a total method efficiency of 61%, determined by running a known concentration of [^{14}C]benzene through the sampling method.

In expt. 1, rotifers (60/mL) were given a single initial dose on day 1 and left in the dosed pans for 8 d. The contents of two replicate pans for each concentration of benzene, 0.1 and 1.0 μL/L, were monitored daily for ^{14}C activity in the water column and in the rotifers.

In expt. 2, rotifers (20/mL) were exposed to benzene concentrations at relatively constant levels of 0.1 or 1.0 μL/L for 9 d. Because about 70% of the [^{14}C]benzene volatilizes in 24 h (Eldridge and Echeverria 1977), concentrations remaining 24 h after dosing were brought up to the target level by adding [^{14}C]benzene solution equivalent to the amount volatilized. ^{14}C activity in the water was measured daily with the scintillation counter. During the second experiment, the actual concentration of benzene was determined every 2 d with a gas chromatograph for the two replicates of each concentration.

Results — In experiment 1, 80% of ^{14}C was lost from the water to the air, pan surfaces, and rotifers at both initial concentrations of [^{14}C]benzene after 2 d. However, ^{14}C compounds never completely disappeared from the aqueous phase (Fig. 1).

In experiment 2, [^{14}C]benzene was administered to maintain an actual level of ^{14}C activity in the seawater of 0.12 (±0.04 μL/L) and 1.1 (±0.5 μL/L) (Fig. 1) for 9 d. Actual concentration of benzene monitored by the gas chromatograph (GC) was compared to ^{14}C activity monitored by the scintillation counter. On day 1, measurements by both techniques corresponded to the target concentrations of benzene; however, by day 5, the GC failed to detect any benzene in the system while the scintillation counter was showing ^{14}C activity. [^{14}C]benzene solution was added to the experimental pans to maintain the concentration of [^{14}C]-benzene monitored by the scintillation counter. For the duration of these experiments the presence of benzene and of ^{14}C activity were not correlated. Water samples from a pan with a benzene concentration of <0.01 μL/L (GC) and a ^{14}C count corresponding to 0.8 μL/L were sent to an analytical laboratory. Method C (Taras et al. 1971), a standard method for the determination of phenols, indicated that all ^{14}C activity (0.8 μL/L) was due to phenolic compounds. This method includes possible interference from benzene; however, no benzene was detected by GC with a sensitivity of 0.01 μL/L.

Adsorption was measured by taking water samples from the cleaned and refilled pans on day 9, and showed ^{14}C activity of 0.001 μL/L. The only source of ^{14}C would be leaching from the container walls into solution.

In expt. 1, the rotifers accumulated ^{14}C compounds rapidly (Fig. 1), reached an asymptote by day 5, and were maintained for the duration of the experiment. Final biomagnification of ^{14}C compounds in the rotifers on day 8 were 10^2 times, at 0.1 μL/L, and 10^3 times at 1.0 μL/L, the levels in the water. The rotifers accumulated and maintained a high level of ^{14}C compounds which did not appear to be depurated while in the treated water.

In expt. 2, the rotifers under chronic exposure accumulated a maximum of ~10^4 times the initial concentration in the water. A peak in the rotifers was reached on day 6 during which time the benzene (GC) was not present in the water.

Both singly (initial dose) and chronically (constant dose) treated rotifers accumulated ^{14}C compounds to high concentrations. The data suggest that there is a limit to the amount of ^{14}C compounds a rotifer is able to store, with a state of equilibrium being reached between the water and the rotifers. ^{14}C compounds never completely left either system during the 8- or 11-d experimental periods.

Discussion — The presence of phenols in the experiments, instead of benzene, indicates some type of chemical degradation. The stability of benzene and the short duration of these experiments would eliminate photochemical oxidation occurring at any significant level. Possibly the benzene was metabolized by bacteria. Biodegradation of benzene has been demonstrated with in situ marine microbes (Lee and Ryan 1976), specifi-

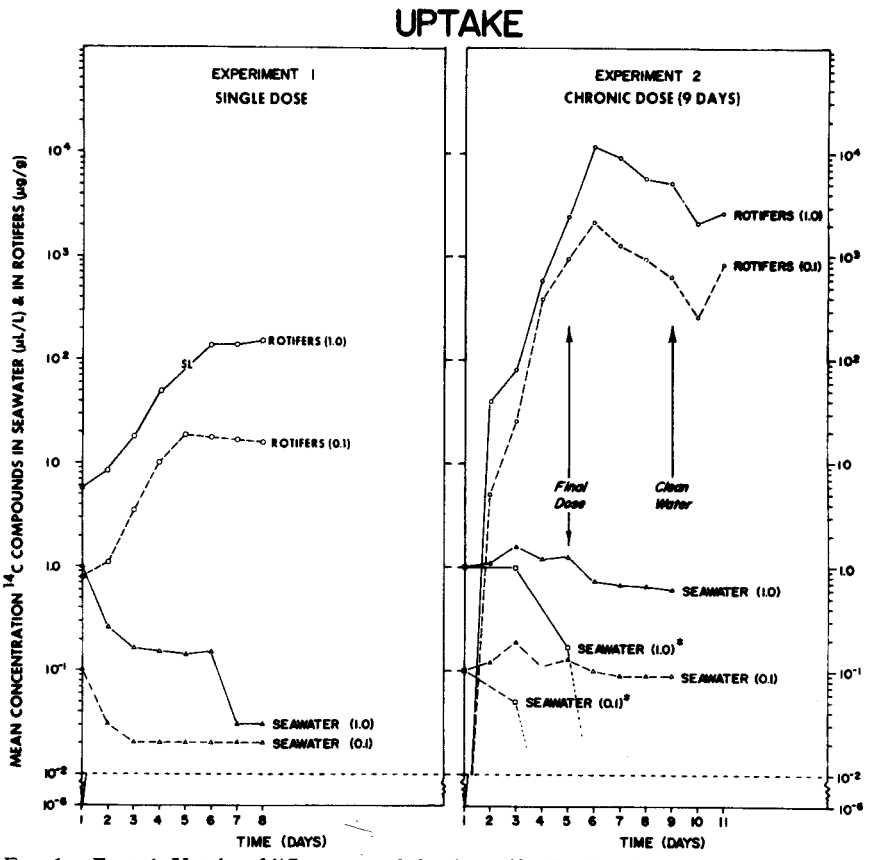

FIG. 1. Expt. 1. Uptake of ^{14}C compounds by the rotifer *B. plicatilis* and the corresponding concentration in the seawater. Expt. 2. Uptake of ^{14}C compounds by *B. plicatilis* under chronic exposure, and the corresponding concentration in the seawater. Values in the seawater measured daily on a scintillation counter and on days 1, 3, and 5 on a gas chromatograph (GC). *GC analysis, concentration of benzene approaches 0 between measurements; solid lines are 1.0 µL/L; broken lines are 0.1 µL/L.

cally the bacteria *Pseudomonas putida* (>1.0 µm long) and *Nocardia* sp. (>1.0 µm long) (Gibson 1976). It is not known whether any marine microbes capable of benzene degradation were present in the experimental pans. To determine if ^{14}C activity was bound to bacteria, a subsample of water from all pans was filtered through 0.45-µm Millipore filter paper and analyzed on the scintillation counter. ^{14}C activity for unfiltered water corresponded to that of the filtered water samples measured on the same day. Therefore, ^{14}C was not bound up in any particle or bacteria larger than 0.45 µm. If the benzene was degraded by bacteria, it was not bound to them.

Biodegradation of benzene by the experimental animals is also a possibility; however, hydrocarbons stored for long periods indicate the inability of animals to metabolize them (Lee 1975). During these experiments, rotifers accumulated and maintained concentrations of benzene and phenolic compounds many times the exposure level. It is possible that the rotifers were metabolizing benzene into phenols, but because ^{14}C compounds were retained by them, the effect is still the same: rotifers accumulated and retained concentrated amounts of ^{14}C compounds during the experimental time frame. Depuration may occur in clean water over a longer time than tested.

Herring larvae exposed to ^{14}C compounds through water accumulated 2 times the concentration in the water, increasing to 3.2 times when fed the rotifer (Eldridge et al. 1978). The ability of the rotifer to accumulate benzene results in the availability of a higher concentration of benzene and related compounds to feeding larval fish, and would increase the possibility of adverse effects. Larval herring showed alterations in their metabolic process when exposed to benzene at 0.02–2.3 µL/L (Eldridge et al. 1977). Juvenile herring assimilate labeled hydrocarbons in the stomach tissue and lipids (Whittle et al. 1977), with unknown conse-

quences. Depuration of hydrocarbons in fish does occur, and depuration of ^{14}C compound in rotifers is an untested possibility. However, rotifers accumulate high concentrations of ^{14}C compounds, larval herring accumulate ^{14}C compounds when feeding on rotifers, and there are metabolic changes in larvae. Just because the fish can metabolize ^{14}C compounds does not eliminate possible adverse effects when both the fish and its food are exposed to the hydrocarbon.

Acknowledgments — I thank Sid Korn for aid in the spectrophotometry method, Vance McClure for suggestions in designing a concentrating apparatus for rotifers, and Jeannette Whipple (formerly J. Struhsaker) for her assistance, support, and critical guidance of this work.

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