Metabolic Alteration and Excretion of Anthracene by *Daphnia pulex*[1]

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) constitute a series of compounds, some of which are potent carcinogens, which have been detected in natural waters throughout the world. Major sources are aqueous effluents from high-temperature industrial pyrolysis processes, including coke production (ANDELMAN and SUESS 1970). Coal liquefaction, which may provide substantial quantities of synthetic fuel within 15 years, may also release PAH in wastewater streams (GUERIN 1975).

Because PAH have been demonstrated to concentrate to high levels in tissues of aquatic organisms (SHIMKIN et al., 1951; SCACCINI-CICATELLI 1966; STEGEMAN and TEAL 1973), a potential exists for biomagnification within food webs to levels hazardous both to fish and to human consumers of aquatic organisms. CORNER (1975) has suggested that uptake by filter-feeding organisms may constitute a major mechanism of entrance of PAH to aquatic food chains. In this laboratory, we have demonstrated the ability of several invertebrates to concentrate anthracene, a three-ring PAH compound, from both dissolved and particulate forms. In this paper we present further investigations of the kinetics of excretion and metabolic alteration of anthracene by a representative zooplankter (Daphnia pulex) to evaluate the effect of excretory processes on the potential for bioaccumulation of PAH compounds in aquatic organisms.

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METHODS AND MATERIALS

Stock cultures of Daphnia pulex were maintained at 23°C in 7.6-liter laboratory aquaria in spring water; homogenized and filtered "Trout Chow" was added twice weekly (GEHRS 1972). Four- to six-day-old animals were isolated for experiments.

A. Excretion and uptake experiments

Several hundred D. pulex were incubated at 23°C in 4000 ml of membrane-filtered spring water to which approximately 50,000 dpm of carrier-free 9-^{14}C-anthracene (32 mCi/mmole; Amersham-Searle) in 20 µl of acetone had previously been added. Two sets of incubation conditions were utilized: one without food present, and one to which autoclaved Enterobacter cells (grown in one percent Difco Bacto-Peptone medium) were added to achieve an initial concentration of 10^6 cells/ml. After 16 hr the animals were removed by decanting through a nylon net and transferred to 4000 ml of unlabelled spring water. At intervals 200 ml of water and samples of 20 animals were removed. Activity in each organism sample was determined by liquid scintillation counting after washing in 2 ml of distilled water, homogenizing in 1 ml acetone in 13 x 80 mm tissue-grinding tubes, and centrifuging (1000 g x 2 min) to remove debris. Concentrations of total ^{14}C in water samples were determined by triple extraction into hexane followed by liquid scintillation counting.

Uptake data were obtained by removing Daphnia and water samples at intervals from similarly treated 50-ml beakers containing 30 ml of water and approximately 3000 dpm of ^{14}C-anthracene (without food added), and extracting and counting ^{14}C as outlined above.

B. Isolation and quantitation of metabolites

Acetone extracts of D. pulex (prepared as above) were applied in 1-cm spots to nonactivated silica gel thin-layer plates (20 x 20 cm), which were than developed in either 9:1 or 19:1 v/v benzene-ethanol (SIMS 1970). One-centimeter bands were removed by scraping, and ^{14}C was extracted with ethanol and quantitated by liquid scintillation. Water samples (100 ml) were extracted with 50-ml ethyl acetate portions, each of which was dried over calcium sulfate and concentrated by rotary evaporation. Residues were redissolved in 1 ml of tetrohydrofuran, chromatographed, and analyzed as above.

A. Uptake

In each of two trials Daphnia rapidly accumulated ^{14}C (Fig. 1) until after several hours an equilibrium with the water

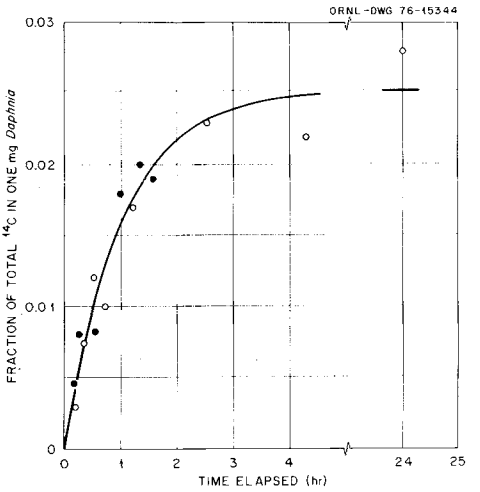

Fig. 1. Uptake of dissolved 9-^{14}C-anthracene by Daphnia pulex. Open and solid circles indicate replicate experiments. Initial anthracene concentration ≈ 0.02 µg/liter. T = 23°C. Curve fit by nonlinear least-squares regression.

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was reached (equilibrium Daphnia concentration: 760 times water-^{14}C concentration). The data are describable by a first-order approach to equilibrium of the form (after SÖDERGREN and SVENSSON 1973):

$$C_t = C_{ss}(1-e^{-kt})$$

where concentrations of ^{14}C in Daphnia after t hours and at steady-state (C_t and C_{ss}, respectively) are expressed as fractions of the total ^{14}C per mg Daphnia. The best-fit value for the rate constant k is 1.01/hr, while the best-fit value for C_{ss} is 0.0252/mg D. (both determined by nonlinear least-squares analysis).

B. Excretion

Levels of ^{14}C remaining within D. pulex after transfer to fresh water are shown (as fractions of the amount initially present) in Fig. 2. In both experiments, 30 to 35% of the total ^{14}C

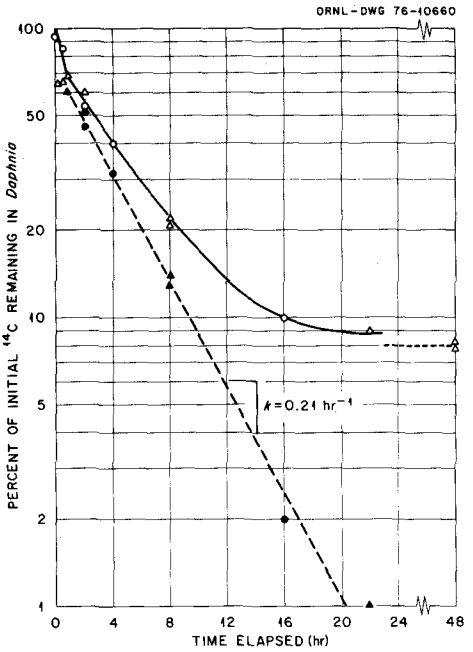

Fig. 2. Excretion of ^{14}C from Daphnia after 16-hr incubation with ^{14}C-anthracene and subsequent transfer to clean water. Open circles: after incubation with suspended Enterobacter sp. cells (10^6/ml); open triangles: after incubation without food present. Solid circles and triangles are 8% less than corresponding open data points, and represent approach to apparent equilibrium position of 0.08 of total initial body burden. T = 23°C. Data treatment after LEAN (1973); curves fitted by eye.

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was rapidly released. While the initial release process appeared to be complete after 5 to 10 min for starved animals, about 50 min were required for D. pulex which had been fed Enterobacter cells.

After the initial rapid release, the release rates slowed considerably in both experiments; in both cases levels within animals asymptotically approached 8% of the initial level. The second excretion process, which in each case resulted in the release of roughly 60% of the total body burden, followed a first-order approach to the asymptote (LEAN 1973) with a half-life of 3.3 hr (k = 0.21/hr).

C. Metabolism

To quantitate the rate of metabolite excretion, 200-ml water samples were removed at intervals, extracted, and chromatographed. Results (Table 1) showed that after a 2-hr lag period, a water-soluble ^{14}C-labelled compound (which traveled 0.65 the distance of ^{14}C-anthracene on TLC plates) appeared in the water and steadily increased in quantity during the course of the experiment. The excretion rate was 0.0016/mg D./hr (determined by nonlinear least-squares regression). No similarly positioned ^{14}C-labelled compounds were observed in spring water incubated without Daphnia.

DISCUSSION

A. Compartmentalization of anthracene

Although the kinetics of uptake of ^{14}C-anthracene by Daphnia fit a simple two-compartment model (consisting of ^{14}C-anthracene in water and in Daphnia), excretion data and isolation of an excreted metabolite suggest that the situation is more complex. At least three internal compartments within Daphnia exist (Fig. 2): a rapidly eliminated compartment containing about 30% of the internal ^{14}C "pool," a more slowly eliminated compartment containing 60%, and a tightly bound residue of 8%.

TABLE 1

Excretion of water-soluble metabolites of ^{14}C-anthracene by Daphnia pulex

Time elapsed after ^{14}C-anthracene addition, hr	Fraction of total ^{14}C in form of water-soluble metabolites
0	< 0.002
0.17	< 0.002
0.5	< 0.002
1.0	< 0.002
2.0	< 0.002
4.0	0.008
24	0.045
48	0.040
96	0.22
168	0.21

Data points fitted by least-squares regression to the equation: $C(\text{metabolite})_t = 1\text{-}e^{-k(t-2)}$. Computed value of k is 0.00160/hr ($r = 0.91$).

Accurate estimation of the rate of the initial excretion process from the data is unfeasible, due both to the rapidity of the process and the few data points available. The data indicate, however, that ^{14}C release was more rapid from starved animals than from animals which had been feeding, and whose guts presumably were full. The gut passage time of D. magna is 40 to 45 min (RIGLER 1961) at 20°C, while that of D. rosea is only 7 min (BURNS and RIGLER 1967); presumably the gut passage time of D. pulex (which is intermediate in size between the two other zooplankton) would be intermediate between the two. The 50 min required by this initial removal process from feeding animals is thus consistent with the interpretation that the first process is gut elimination. The rapid elimination observed from unfed animals may represent passage of water through the food groove and gut, causing rapid desorption of ^{14}C-anthracene which had been taken up from the dissolved phase.

The two-step excretion and approach to an asymtote observed for D. pulex is quite similar to results of excretion of ^{14}C-napthalene from marine copepods (Fig. 3) (CORNER, unpublished data). Each of the copepods released between 45 and 65% of the total ^{14}C present within 1.5 to 10 hr (compared to 35% and less than 1 hr, respectively, for D. pulex), Each of the three then released ^{14}C slowly, according to a first-order approach to an equilibrium level of between 1 and 5% of the initial amount present (compared to 8% for D. pulex). The close similarities of both size and release rate of the initial compartment suggest that gut elimination may occur in the marine zooplankton as well as in Daphnia.

The relatively non-excretable residual ^{14}C compartment may also be a common phenomenon: both Tubifex worms (SCACCINI-CICATELLI 1966) and oysters (STEGEMAN and TEAL 1973) have been postulated to bind aromatic hydrocarbons irreversibly.

B. Effect of metabolite excretion on anthracene accumulation

Although a considerable fraction of the total ^{14}C in the system was converted to anthracene metabolites over the experimental period, from TLC data only 2% of the ^{14}C within the animals was polar in nature. Apparently metabolically altered ^{14}C-anthracene is immediately excreted; the ^{14}C body burden in the animals, therefore, almost entirely represents unaltered anthracene.

To assess the importance of metabolic alteration and excretion in determining the anthracene body burden in Daphnia, the relative rates of excretion of metabolites and of unaltered anthracene may be compared. When the Daphnia ^{14}C body burden is constant, the rate of influx of ^{14}C-anthracene is equal to the sum of rates of excretion of metabolized and unmetabolized ^{14}C-anthracene. By differentiation of equation (1) the net uptake rate is calculated:

$$\frac{dC_t}{dt} = kC_{ss}e^{-kt} \quad . \tag{4}$$

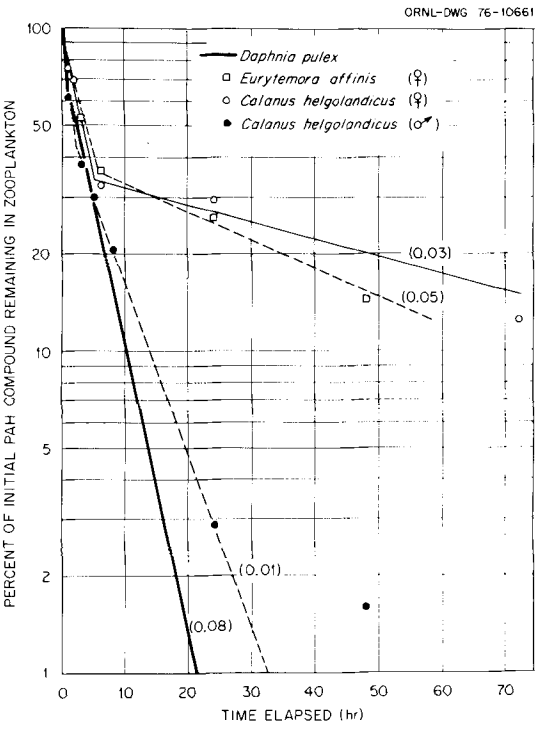

Fig. 3. Excretion of naphthalene by marine copepods. Decimal fractions in parentheses indicate apparent residual equilibrium levels in organisms. Eurtemora affinis data transformed from Fig. 1 in CORNER (1975); Calanus helgolandicus data from CORNER (personal communication; unpublished observations). All data used with permission. Excretion pattern of ^{14}C-anthracene by Daphnia (Fig. 1) included for comparison. Curves fitted by eye.

The influx rate may then be determined at t_o when the rates of ^{14}C-metabolite and ^{14}C-anthracene excretion are both zero:

$$\lim_{t \to o} \frac{dC_t}{dt} = kC_{ss}, \tag{5}$$

or 0.026/mg D./hr. The sum of the two elimination process rates is thus also 0.026/mg D./hr (between 4 and 24 hr when the Daphnia body burden is at steady state). The observed rate of metabolite excretion during this period is 0.0016/mg D./hr -- only 6% of the total ^{14}C outflux rate. Because the rate of metabolite excretion is very small in comparison with the transfer rate of unaltered ^{14}C-anthracene, metabolite excretion apparently only slightly decreases the ^{14}C-anthracene body burden of the animals.

Although D. pulex are able to metabolize PAH compounds, the rate of metabolic excretion is likely to be insufficient to inhibit bioaccumulation by several hundred-fold factors, primarily because uptake of PAH by Daphnia is extremely rapid. A significant potential for bioconcentration of the compounds by fish through zooplankter predation therefore exists. The rate of metabolism of PAH compounds by marine fish (LEE et al., 1972) is more rapid than that by Daphnia, and may be more similar to the rate of uptake; metabolic elimination would then have a greater effect on net bioaccumulation by fish. Metabolic alteration of PAH by zooplankton may be more ecologically important as a mechanism of net degradative removal of PAH from natural waters, than as a mechanism for prevention of biomagnification of the compounds within aquatic food webs.

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