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Bioaccumulation of Carbazoles: A Potential Effluent from Synthetic Fuels[1]

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Aqueous wastes from coal conversion technologies contain several classes of mutagenic and/or carcinogenic organic compounds. The environmental behavior of one such class of compounds, carbazoles, has received little study. In this paper, we report the bioaccumulation potential and kinetics of two members of this class, carbazole and 13 H dibenzo(a,i)carbazole, in the zooplankter, Daphnia pulex. The latter compound has been shown to induce neoplasia in laboratory animals (CHRISTENSEN et al. 1975). Daphnia pulex previously has been used to study the bioaccumulation potential of coal-derived organic compounds (SOUTHWORTH et al. 1978a,b) and provides a simple and rapid test for identifying substances which may accumulate in aquatic biota.

METHODS

The studies were carried out at 21° C ± 1° C in a 3000-ml beaker, using spring water filtered through a Whatman #40 glass fiber filter. Characteristics of the exposure medium were: pH 7.5 to 7.9, total alkalinity ∿ 80 ppm, and dissolved oxygen 6 to 8 ppm. Adult Daphnia, ∿ 0.5 mg wet weight per individual, were placed in the container at an initial density of about 67 animals per liter. Replicate samples of five animals each were randomly selected at periodic intervals, isolated by filtration, and extracted. The test compounds were extracted from the Daphnia by placing five intact organisms in 5 ml of methanol and gently shaking for 30 min. Homogenization and reextraction of the Daphnia indicated that the initial extraction was > 95% complete. Mean wet weight per individual of the test population was estimated by selecting ten organisms at random, isolating them by filtration, and rapidly weighing each individually.

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Analyses of carbazoles in water and methanol extracts were made with a Perkin-Elmer MPF-44 fluorescence spectrophotometer. The method of standard additions was used periodically on individual samples to validate the use of calibration curves, which were constructed daily.

Aqueous concentrations of the test substances used in the study were 65 μg/l for carbazole and 10.5 μg/l for dibenzocarbazole. While the aqueous carbazole concentration remained essentially constant over the course of the experiment, dibenzocarbazole declined to 5.5 μg/l in 54 hr. This necessitated utilization of a two-stage procedure compensating for this decline to elucidate uptake and elimination kinetics (SOUTHWORTH et al. 1978a). In this procedure, an exponential function describing Z(t) was incorporated into equation (1) before solving its integrated form, using a least squares analysis.

Bioaccumulation was described using the simple uptake-elimination model widely used in bioaccumulation studies (NEELY et al. 1974, BRANSON et al. 1975),

$$\frac{dY(t)}{dt} = CZ(t) - kY(t) \quad , \tag{1}$$

where Z(t) = aqueous carbazole concentration at time t after the start of the experiment, (μg/g),

Y(t) = Daphnia carbazole concentration at time t after the start of the experiment, (μg/g) net weight,

C = Daphnia uptake rate coefficient, hr^{-1}, and

k = Daphnia elimination rate coefficient, hr^{-1}.

This model was employed to estimate uptake (C) and elimination (k) coefficient (SOUTHWORTH et al. 1978a).

The equilibrium concentration factor (concentration of carbazole in Daphnia/concentration of carbazole in water) approaches C/k as a limit as $t \rightarrow \infty$, and thus was estimated as the ratio of C to k.

An independent estimation of the elimination rate coefficient was made by placing animals which had attained equilibrium in filtered spring water at 21°C and periodically removing and extracting two five-animal replicates. The elimination rate coefficient (k) was determined from the regression of ln Y(t) vs t.

RESULTS

Near equilibrium concentrations of both carbazole and dibenzocarbazole were attained within the Daphnia by the end of 24-hr exposure. Carbazole was not accumulated to a very high degree, exhibiting an equilibrium concentration factor of 115 ± 3 (Fig. 1). The suspect carcinogen, 13 H dibenzo(a,i)carbazole, was accumulated to a much greater extent, with an equilibrium concentration factor of 712 ± 122 (Fig. 2).

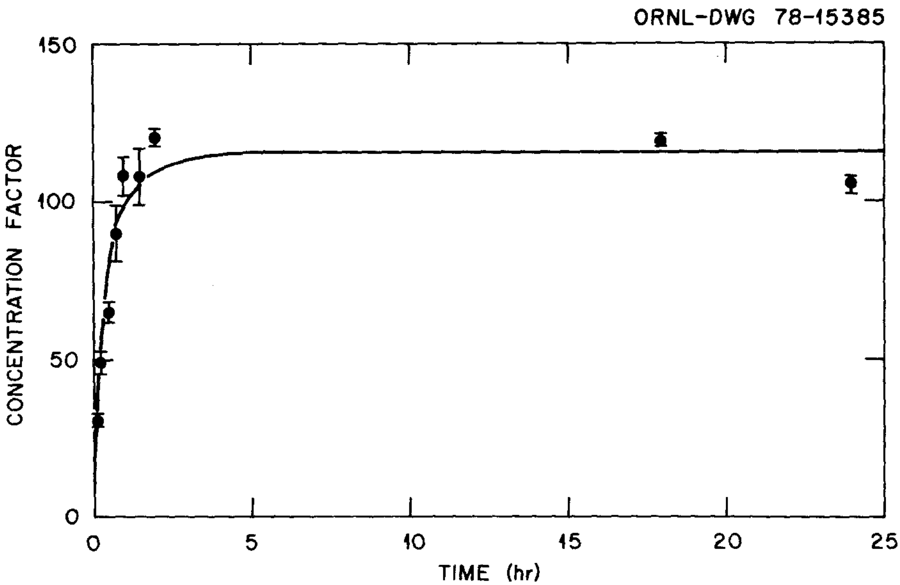

Figure 1. Bioaccumulation of carbazole by Daphnia pulex at 21°C. Concentration factor is concentration of carbazole in Daphnia (wet weight) divided by concentration of carbazole in water. Curve fitted by two-stage least-squares linear regression procedure.

The uptake constant, C, did not vary greatly for the two compounds, with values of 238 ± 17 and 548 ± 60 hr^{-1} for carbazole and dibenzocarbazole, respectively (Table 1). Similar results have been observed for polycyclic aromatic hydrocarbons and azaarenes (SOUTHWORTH et al. 1978a,b). The elimination rate coefficient, k, was considerably lower for the dibenzocarbazole than for the carbazole. The estimates of k obtained using the two different methods do not agree well (Table 1), but nevertheless demonstrate that elimination of both compounds occurs and that their accumulation in Daphnia is a result of a dynamic equilibrium between uptake and elimination (Fig. 3).

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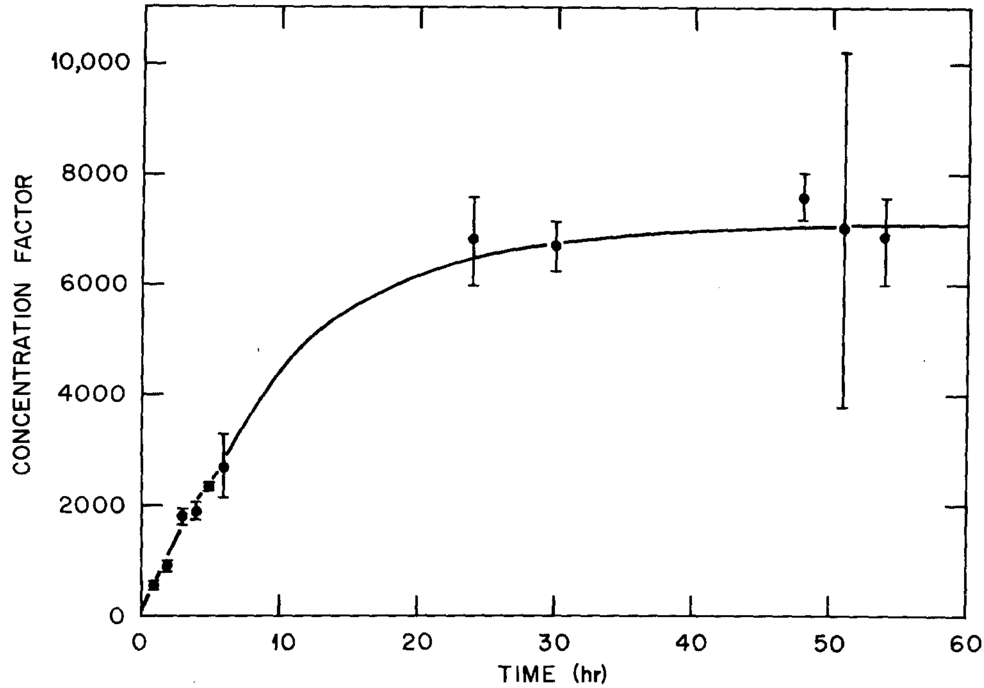

Figure 2. Bioaccumulation of 13 H dibenzo(a,i)carbazole by Daphnia pulex at 21°C. Concentration factor as previously defined. Curve fitted by two-stage linear regression procedure.

TABLE 1

Bioaccumulation coefficients of carbazole and 13 H diabenzo(a,i)carbazole for Daphnia pulex at 21°C. Error intervals are ± 1 S. E.

	Uptake rate C coefficient, hr^{-1}	Elimination rate k coefficient, hr^{-1}	Concentration factor, C/k	Independent elimination rate coefficient, k'
Carbazole	238 ± 17(N=18)	2.06 ± 0.18(N=18)	115 ± 3(N=18)	0.618 ± 0.064(N=18)
13 H dibenzo(a,i)-carbazole	548 ± 60(N=22)	0.077± 0.010(N=22)	7126 ± 122(N=22)	0.117 ± 0.009(N=22)

Estimates of elimination half-lives were about 1 and 6 hr for carbazole and dibenzocarbazole, respectively, when determined by the depuration procedure, while estimates of about 20 min and 9 hr were obtained using the bioaccumulation data. Elimination rates obtained using the depuration method are probably more reliable than those from the bioaccumulation experiment, since elimination and uptake are covariate in the latter technique.

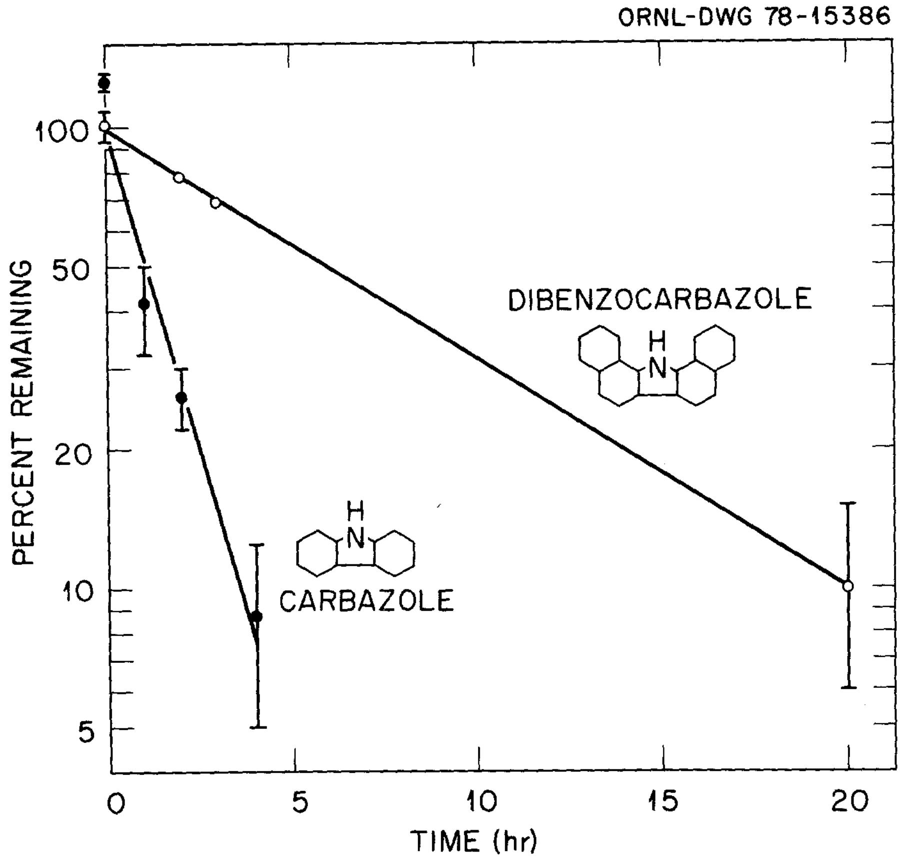

Figure 3. Elimination of carbazole and 13 H dibenzo(a,i)carbazole by Daphnia pulex at 21°C.

The observed bioaccumulation potential of 13 H dibenzo(a,i)carbazole is within an order of magnitude of the levels of bioaccumulation observed in Daphnia for substances such as DDT, aldrin (JOHNSON et al. 1971), and methyl mercury (HUCKABEE et al. 1975). While bioaccumulation potential of dibenzocarbazole is indeed substantial, the hazard represented by bioaccumulation for any substance cannot be evaluated without knowledge of the magnitude of inputs to the aquatic system, the persistence of the substance in the system, its accumulation and metabolic fate in higher organisms, and an assessment of the possible health threat posed by the resulting concentrations of the substances in food ingested by humans. The bioaccumulation data resulting from this study point out a need for evaluating high molecular weight polyheterocyclic substances in synfuel products and effluents with respect to source magnitude, environmental fate, and toxicological properties.

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