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Bioaccumulation of Fossil Fuel Components During Single-Compound and Complex-Mixture Exposures of *Daphnia magna*

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The evaluation of potential ecological consequences from introduction of fossil fuel chemicals into aquatic environments is a complicated process that requires information about physical, chemical, and biological properties of many different types of organic compounds. One concern in aquatic hazard evaluation is the bioaccumulation of these organic components by aquatic biota. Two general approaches are usually used to assess the potential uptake and retention of organic compounds from complex petroleum or bituminous mixtures. In one approach, organisms are exposed to a complex water-soluble fraction or chemical mixture (Roubal et al. 1978; Woodward et al. 1981; Linder et al. 1984), and accumulated tissue levels of specific compound classes are compared. A second approach has been to study the environmental fate of contaminants through single-compound exposures (Vieth et al. 1979; Bishop and Maki 1980). A criticism of the first method is that it fails to account for pathways of individual compounds because of metabolism.. The second approach provides information on fate and effects for only one of many possible components and does not account for potential chemical interactions. Additionally, recent evidence indicates that the single-compound approach to predicting bioaccumulation potential of certain organic compounds may be inappropriate when the constituents are contained within a complex organic mixture, for example, a potential effluent from an oil shale industry (Linder and Bergman 1982). It therefore seems reasonable that competitive interactions for biotic transport may occur among constituents in complex aqueous effluents, particularly if compounds possess similar physical or chemical properties.

We conducted tests with the water flea (Daphnia magna) to compare the bioaccumulation of compounds presented alone with the bioaccumulation of these same compounds when they were presented within a complex coal liquid, water-soluble fraction. Phenol and aniline were used as representative compounds because they are highly soluble, moderately toxic, and common to many fossil fuel liquid products and corresponding wastes. The tests were primarily designed to aid in development of predictive models relating to the transport and fate of components from complex mixtures in aquatic biota.

METHODS AND MATERIALS

Adult daphnids were obtained from cultures reared at our laboratory. During exposure, daphnids were held in 600-mL glass beakers containing 500 mL of well water at 20°C. Initial loading rates were 60 to 75 daphnids for each of four replicate beakers at each treatment. Five daphnids from each treatment were removed for uptake counts after 1, 2, 4, 8, 16, and 24 hours of exposure. Remaining daphnids were transferred by plankton netting to fresh well water at 24 hours, and removed for depuration counts at 25, 26, 28, 32, 40, and 48 hours. The daphnids were fed prior to test initiation only.

The water-soluble fraction (WSF) used in all tests was generated using a slow-mix procedure and an oil-to-water ratio of 1:99 (Dauble et al. 1982). The coal liquid was a 2.9:1 blend of middle to heavy distillate produced by the Solvent Refined Coal (SRC II) process. It was obtained from a pilot plant at Fort Lewis, Washington, and stored at 4°C until use. The WSF was chemically characterized for overall composition, and phenolics were found to comprise about 90% of the total organic carbon (TOC). The major phenolic constituents were phenol, 15%; cresols, 37%; C_2 phenols, 20%; and C_3 phenols, 9% (Dauble et al. 1982). Alkyl anilines were the major nitrogen-containing compounds (Felice 1982). Test organisms in the single-compound-plus-WSF treatment were exposed to nominal concentrations of 0.5 mg/L TOC.

All radiolabeled chemicals were obtained from Pathfinder Laboratories, St. Louis, Missouri, and were >98% pure. Specific activities were as follows: ^{14}C aniline as aniline sulfate, 9.56 µCi/mM; ^{14}C phenol, 11.61 µCi/mM. Total phenol concentrations were adjusted to equivalent levels as follows: for single-compound exposure, 696 µg/L of ^{14}C phenol plus 80 µg/L as unlabeled phenol; for single compound with WSF, 696 µg/L as ^{14}C phenol plus 86 µg/L estimated as WSF phenol. Total aniline concentrations in the two treatments were for single-compound exposure, 484 µg/L as ^{14}C aniline; for single compound plus WSF, 484 µg/L as ^{14}C aniline plus an estimated 6.5 µg/L as WSF aniline. All concentration values reported during testing are based on measured radioactivity.

Water samples were collected at 0, 1, 2, 4, 8, 16, and 24 hours after test initiation. The 1-mL samples were diluted with PCS scintillation fluid (Amersham Searle) and counted using liquid scintillation spectrometry. All samples were corrected for quench effects.

Five daphnids were removed by pipette at each sample interval for tissue analysis. They were placed on filter paper, rinsed four or five times with distilled water, and air-dried under a light vacuum. The daphnids were then placed into scintillation cocktail and sonicated for 1 hour to aid dissolution of the carapace. Liquid scintillation was used to count the samples for total radioactivity. Eight replicate groups of five daphnids each were blotted dry on tissue paper and weighed (wet weight), then dried at 40°C for 24 hours to obtain mean dry weight. Determination of ^{14}C bioconcentration factors was based on micrograms equivalent ^{14}C per gram dry weight of daphnids. The mean wet weight of individual daphnids was 2,143 ± 76 µg, and the dry weight averaged 146 ± 4 µg.

Kinetic model theory and nonlinear least square techniques were used to obtain estimates of uptake and depuration rates and bioconcentration factors (BCFs) for tests with daphnids. A two-compartment (daphnid and water) closed system was used as the model for exchange of the radiolabeled compound (Hamelink 1977) to obtain simultaneous estimates of uptake and depuration from the uptake phases of the experiment. Independent estimates of depuration rates from the depuration phase data were also used to estimate BCFs and ^{14}C half-life values. Differences in uptake and depuration phase responses of the single compound and the compound plus WSF were tested using a general linear test (Neter and Wasserman 1974).

RESULTS AND DISCUSSION

Mean water concentrations of phenol in the phenol-only exposures with daphnids declined slightly from 696 µg/L at 0 hours to 634 µg/L at 24 hours. A similar decline was observed in the phenol-plus-WSF exposures with daphnids; maximum concentrations of 702 µg/L were observed at 2 hours and the lowest concentration of 616 µg/L was noted after 8 hours of exposure. Small amounts of radioactivity were detected during depuration and may have resulted from excretion of absorbed phenol by the daphnids. Mean ^{14}C concentrations in the water after 24 hours depuration were less than 2 µg/L for both treatments.

Concentrations of parent compound plus metabolites in daphnids for the phenol-only treatment increased rapidly during the 24-hour uptake period and were greater than for the phenol-plus-WSF treatment at all time intervals after 1 hour (Figure 1a). BCFs estimated from ^{14}C activity and using dry-weight conversions were 1,375 and 876 for phenol-only and phenol-plus-WSF exposures, respectively. Estimates of BCF based on kinetic model theory were lower (Table 1). Significant differences ($\alpha \leq 0.001$) were detected in the uptake rate constants of parent compound plus metabolite between the single-compound and compound-plus-WSF exposures. However, no significant difference was found for the depuration rate constant. Estimated half-lives for the phenol-only and phenol-plus-WSF treatments were 8.0 hours and 12.1 hours, respectively.

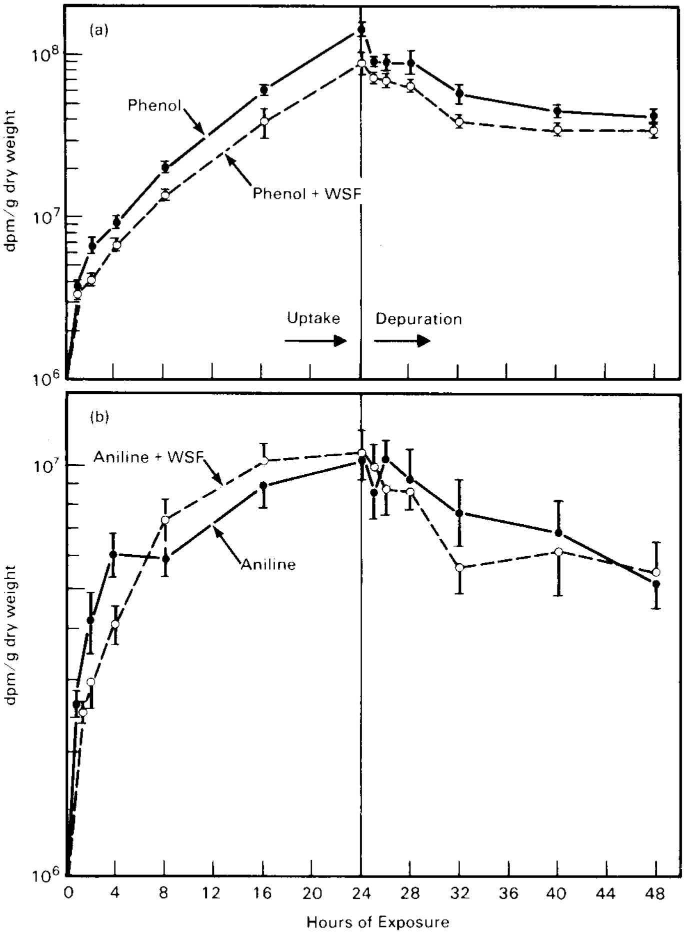

Figure 1. Comparison of bioaccumulation in daphnids during single-compound and complex-mixture exposures. values given as ± S.E., N = 4.

Mean concentrations of aniline in the aniline-only exposures with daphnids declined from a maximum of 484 µg/L after 1 hour of exposure to 449 µg/L after 24 hours. Concentrations in the aniline-plus-WSF treatment were similar; mean ^{14}C activity ranged from 466 µg/L at 1 hour to 438 µg/L after 8 hours of exposure. Negligible radioactivity was detected in water during depuration; aniline concentrations at 48 hours were less than 1 µg/L.

Concentrations of parent compound plus metabolites in daphnids were similar at each of the sample intervals for both treatments

Table 1. Estimates of uptake and depuration rate constants and bioconcentration factor (BCF) for daphnids.

Compound	Uptake Rate Constant (K1)(a)	Elimination Rate Constant (K_2)		Bioconcentration Factor
		Based On Uptake Phase	Based On Elimination Phase(b)	Based On Elimination Phase
Phenol	15.751 ± 2.089(c)	-0.089 ± 0.009	0.057 ± 0.011	277 ± 66
Phenol + WSF	10.744 ± 3.398	-0.083 ± 0.021	0.045 ± 0.009	237 ± 88
Aniline	15.052 ± 2.959	0.226 ± 0.055	0.025 ± 0.009	593 ± 234
Aniline + WSF	10.701 ± 2.729	0.137 ± 0.031	0.023 ± 0.009	469 ± 227

(a) Uptake rate constants estimated from the equation $C_d = (K_1/K_2)C_w(1 - e^{-K_2 t})$, where K_1 is the uptake rate constant, K_2 is the depuration rate constant, C_w is the dpm of ^{14}C labeled compound in the water at steady state. C_d is compound concentration in D. magna, and t is time.

(b) Elimination rate constants estimated from the equation $C_d = K_1 e^{-K_2 t}$, where K_1 is the concentration in D. magna at time ϕ.

(c) All values are mean ± 1 standard deviation.

with aniline (Figure 1b). Based on concentrations of ^{14}C in daphnids and exposure water, bioconcentration factors at peak uptake (24 hours) for aniline-only and aniline-plus-WSF treatments were 74 and 76, respectively. These values are similar to those obtained by kinetic model theory when only the uptake data are used, but are considerably less than BCF estimates derived from the elimination phase data (Table 1). There was no significant difference ($\alpha > 0.05$) in either the uptake or depuration rate constants between the two treatments. Estimated ^{14}C half-lives were 26.5 hours for both the aniline-only and aniline-plus-WSF exposures.

The differences in observed BCFs may be attributable to the chemical composition of the WSF. Since phenolic compounds comprised nearly 90% of the soluble components, differences in uptake of phenol could be due to competitive interactions among similar molecules for uptake and absorption by the organisms. The presence of chemicals of similar size and form may have also resulted in a saturation of hydrophobic binding sites. Depuration rates were similar for all cases, suggesting that mechanisms of elimination were unaffected by prior exposure to the complex mixture.

Since metabolites were not characterized, the relative amounts of parent compound and metabolites in daphnid tissues are unknown. However, it is expected that some portions of the test compounds were metabolized. In studies with the phytoplankter Scenedesmus quadricauda, Hardy et al. (1984) reported that significant quantities of aniline and phenol were biotransformed to metabolites in 24 hours. Additionally, Call et al. (1980) found that fathead minnows (Pimephales promelas) retained only 0.7% of ^{14}C phenol as parent compound after 28 days of exposure. Identification of metabolites would also be useful data for the elimination phase since differential elimination of parent compound and metabolites has been documented for petroleum-derived hydrocarbons in fish (Melancon and Lech 1978).

Differences in potential for bioaccumulation of phenol plus metabolites in complex mixtures are not consistent with estimates of bioaccumulation of phenol as determined by measured octanol/water coefficient values (Table 2). BCF estimates of phenol, based on $\log_{10} K_{OW}$ values, would be similar under both exposure conditions and would be much lower than our laboratory-determined values. Our results also contrast with studies by Veith et al. (1979) who, in exposing fathead minnow to individual and mixed solutions of p,p'DDE and heptachlorepoxide, obtained similar BCFs for both. However, these investigators postulated that uptake of chemicals in mixtures would only be independent of other chemicals if that metabolism of the organism was not significantly altered.

Table 2. Comparison of octanol/water partition coefficients derived for aniline and phenol as single compounds and in aqueous extracts of an SRC II liquid.

Compound	$\text{Log}_{10}\ K_{ow}^{(a)}$	$\text{Log}_{10}\ K_{ow}$ in $\text{WSF}^{(b)}$
Phenol	1.46	1.43
Aniline	0.90, 0.98	0.96

(a) From Leo et al. (1971).
(b) From Thomas (1984)

The effects of multiple contaminants on subsequent metabolism of chemicals of concern were recently studied by Fair and Sick (1983), who reported that simultaneous exposure of black sea bass to napthalene and cadmium was an important factor in determining synergistic or antagonistic effects on accumulation. Thus, the entire spectrum of environmental chemicals can be expected to influence uptake, storage, and elimination mechanisms within an organism.

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