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Uptake and Fate of Phenol and Aniline in Rainbow Trout and Daphnids During Single-Compound and Complex-Mixture Exposures

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October 1984

Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
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UPTAKE AND FATE OF PHENOL AND ANILINE IN RAINBOW TROUT AND DAPHNIDS DURING SINGLE-COMPOUND AND COMPLEX-MIXTURE EXPOSURES

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SUMMARY

Studies were conducted of the potential for uptake and mobilization of phenol and aniline when presented as single compounds to the biouptake of these compounds within a complex water-soluble fraction (WSF) of a coal liquid. Estimated bioconcentration factors (BCF) of phenol-only exposures differed from BCFs obtained in the presence of the WSF. Differences in uptake could be due to competitive interactions among similar molecules for uptake and absorption, since phenolic compounds comprised nearly 90% of the soluble components in the complex mixture. Observed differences in unextractable ^{14}C residues suggested selective binding of phenol or metabolites to trout tissue storage sites. Differences in potential for bioaccumulation of phenol in complex mixtures were not consistent with estimates of BCF as determined by measured octanol/water coefficient values. In contrast to phenol, presence of coal-liquid water solubles did not significantly influence either the uptake or elimination of ^{14}C aniline by daphnids or trout. Identification of metabolites would provide useful information on potential differences in biotransformation and elimination mechanisms in complex organic mixtures.

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INTRODUCTION

Synthetic fossil fuel production processes are currently being developed to augment U.S. domestic supplies of liquid fuel. These activities result in an increased potential for environmental contamination through manufacture, transportation, or waste disposal operations. Fossil fuel liquids contain organic aromatic compounds with oxygen and nitrogen functionality, many of which possess toxic or mutagenic properties (Strand and Vaughan 1981). The evaluation of potential environmental 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 of aquatic hazard evaluation is the uptake and retention of these organic components by aquatic biota. Two general approaches can be used to assess the potential uptake of organic compounds from complex petroleum or bituminous mixtures. In one approach, organisms are exposed to a complex water-soluble fraction (Roubal, Stranahan and Malins 1978; Woodward, Mehrle and Manck 1981), and accumulated tissue levels of specific compound classes are compared. A second approach used by researchers has been to study the environmental fate of contaminants through the use of single-compound exposures (Vieth, DeFoe and Bergstedt 1979). A criticism of the first method is that it fails to account for pathways of individual compounds because of metabolism, conjugation, and/or degradation; the second approach provides information on fate and effects for only one out of many possible components at a time. 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) and rainbow trout (Salmo gairdneri) to compare the potential for uptake and mobilization of single compounds presented alone with the potential when those same compounds 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 protocol development relating to the transport and fate of components from complex mixtures in aquatic biota.

EXPERIMENTAL DESIGN

Juvenile rainbow trout (150 to 950 mg) were obtained from parent stock reared at our laboratory. For each test series, static exposures were initiated by placing 24 fish in each of two 60-L glass aquaria containing 50 L of well water. Three fish from each treatment were removed after 4, 8, 24, 48, and 72 hours of exposure for uptake counts and tissue extractions. At 72 hours the remaining fish were transferred to clean well water for depuration. Three fish from each treatment were removed for elimination counts and tissue extractions after 76, 80, and 96 hours of exposure. Fish were not fed during the 96-hour test.

Adult daphnids were obtained from cultures reared at our laboratory. 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. Daphnids were fed prior to test initiation only.

EXPOSURE METHODS

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 TC.

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 (trout exposures), 6.8 µCi/mM; ^{14}C phenol (daphnid exposures), 11.61 µCi/mM.

Trout Exposures

For the phenol-only and phenol-plus-WSF treatments with rainbow trout, 200 µCi of ^{14}C phenol was added to each exposure aquaria. Total phenol concentrations in exposure aquaria were adjusted to the same concentration as follows: single-compound exposure, 55 µg/L as ^{14}C phenol plus 75 µg/L nominal as cold phenol; single compound with WSF, 55 µg/L as ^{14}C phenol plus 75 µg/L nominal as WSF phenol.

For the aniline and aniline-plus-WSF treatments with rainbow trout, 200 µCi was added to the aquaria. Total aniline concentrations in exposure aquaria were for single-compound exposure, 32 µg/L as ^{14}C aniline; and for single compound with WSF, 32 µg/L as ^{14}C aniline plus 6 µg/L remaining as WSF aniline. Since aniline concentration in the coal liquid WSF contributed only an estimated 6 µg/L, no adjustment was made in the single-compound exposure to equalize aniline concentrations between treatment aquaria.

Daphnid Exposures

Total activity of ^{14}C phenol in daphnid exposure water at test initiation was ~115,000 dpm/mL. 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 cold phenol; for single compound with WSF, 696 µg/L as ^{14}C phenol plus 86 µg/L nominal as WSF phenol.

Total activity of ^{14}C aniline in daphnid exposure water at test initiation was ~145,000 dpm/mL. 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 estimated 6.5 µg/L as WSF aniline.

ANALYTICAL CHEMISTRY

During the exposures, 1-mL samples of the water were pipetted into liquid scintillation vials. For the rainbow trout test series, samples of the water were collected, in duplicate, at 0, 4, 8, 24, 48, and 72 hours after initiation of each exposure. Daphnid 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.

Three fish were collected at 4, 8, 24, 48, 72, 76, 80 and 96 hours after test initiation for measurements of whole body uptake. Each fish was rinsed in distilled water, blotted dry, and placed in a liquid scintillation vial. Samples were then stored at -20°C prior to chemical analysis. Whole fish were thawed and weighed into 25-mL Corex® centrifuge tubes, and 2 mL of acetone (Burdick and Jackson, distilled in glass) was added to each centrifuge tube. The samples were homogenized for 30 sec using a Tekmar® Tissumizer. The homogenized samples were centrifuged (5,000 rpm for 5 minutes) and the supernatants transferred to a 5-mL sample vials. Each tissue pellet was subjected to a second homogenization (2 mL of acetone), and a 200 µL aliquot from the combined extract of each whole fish was counted using liquid scintillation.

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The pelleted tissue residues were dried in a oven at 60°C for 2 hours. Weighed samples were transferred to 2-mL reaction vials to which was added 1 mL of 4 N NaOH. The samples were heated at 60°C for 4 hours and cooled to room temperature. They were shaken occasionally during the digestion process. Liquid scintillation techniques were used to count Aliquots of each sample (~200 µL). A calibration curve was prepared that related dry residue weight to wet weight, in order to allow comparison of acetone-soluble and insoluble forms of ^{14}C activity. Thus, the ^{14}C activity in the base digested tissue could be translated into a ^{14}C activity on a wet-weight basis. Wet weight of the fish used to generate the calibration curve ranged from 250 mg to 1250 mg.

Five daphnids for tissue analysis were removed by pipette at each sample interval. 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 ^{14}C activity. 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.

STATISTICAL ANALYSIS

A general linear test (Neter and Wasserman 1974) was used to compare differences over time in the percent of nonextractable material found in trout exposed to the single compound alone to the percent found in the trout exposed to the single compound plus WSF. For each chemical, the regression of nonextractable parent material over time was compared to that for fish exposed to the complex mixture. Since the independent

variable in the regressions was time, tests for serial correlation (Durbin-Watson) were also conducted. The results were not significant (α >0.05).

Kinetic model theory and nonlinear least square techniques were used to obtain estimates of uptake and depuration rates and 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 1975) 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).

COMPARISON OF SINGLE-COMPOUND AND COMPLEX-MIXTURE EXPOSURES

Mean water concentrations in the phenol-only exposure with rainbow trout declined from 7,000 dpm/mL at 0 hours to 5,800 dpm/mL at 72 hours. Concentrations in the phenol-plus-WSF exposures were similar and ranged from 7,800 dpm/mL at 0 hours to 5,900 dpm/mL at 72 hours.

Total uptake of ^{14}C phenol differed when rainbow trout were exposed to ^{14}C phenol alone, as opposed to exposures of trout to ^{14}C phenol in the presence of the SRC II WSF (Figure 1). In the phenol-only exposure, total ^{14}C activity in whole fish tissue increased from a mean value of 41,000 dpm/g at 4 hours after initiation of the exposure, to 223,000 dpm/g at 72 hours. Tissue of fish placed in clean water after 72 hours decreased to 35% of the high mean ^{14}C activity at 96 hours. In the phenol-plus-WSF exposure, ^{14}C activity ranged from a mean value of 41,000 dpm/g at 4 hours after initiation of exposure to 138,000 dpm/g at 72 hours. Body burdens of fish placed in clean water for 24 hours decreased to 43% of the high mean ^{14}C activity observed at 72 hours.

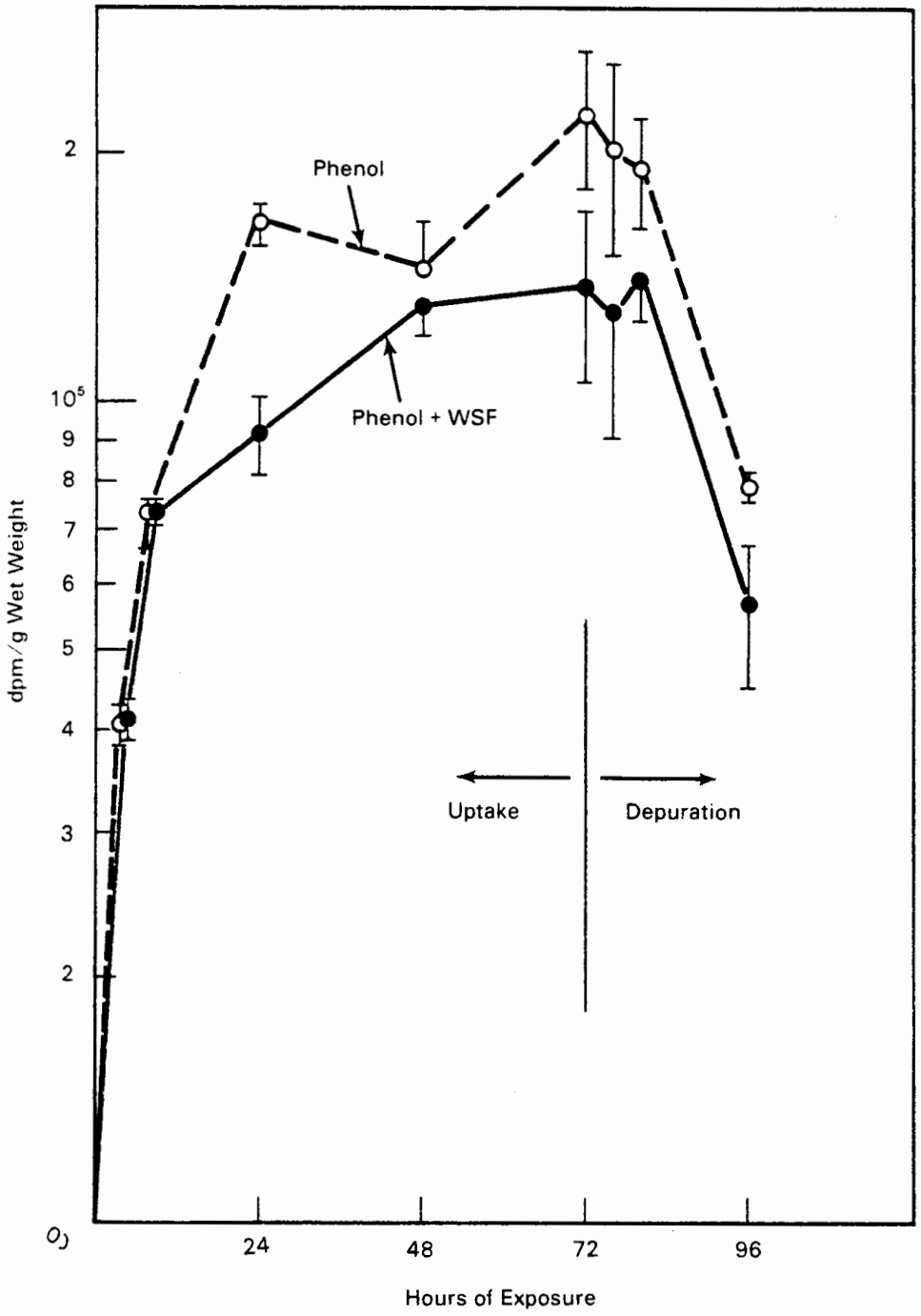

FIGURE 1. Total ^{14}C Activity (Extractable Plus Non-Extractable) in Rainbow Trout Exposed to ^{14}C Phenol and ^{14}C Phenol Plus Water-Soluble Fraction (WSF). All Values Mean ± S.E., N = 3.

Based on concentrations of ^{14}C in whole body fish and exposure water, ^{14}C bioconcentration factors were estimated for accumulation of phenol in rainbow trout exposed to water containing phenol only and phenol plus WSF. The bioconcentration factor at 72 hours for the phenol-only treatment was estimated at 39, and for phenol plus WSF, the value was estimated to be 24.

We also segregated the activity into acetone-extractable and nonextractable fractions in order to gain greater insight regarding the chemical form of the total ^{14}C activity (Table 1). In the phenol-only exposure, from 93% to 96% of the total ^{14}C activity was recovered from

TABLE 1. Comparison of Distribution of Acetone-Extractable ^{14}C Activity in Rainbow Trout Exposed to Treatments of ^{14}C Phenol and ^{14}C Phenol Plus Coal Liquid Water-Soluble Fraction (WSF).[a]

Exposure Time (hr)	% Extractable ^{14}C Activity	
	^{14}C Phenol Only	^{14}C Phenol + WSF
	----------Uptake----------	
4	96.3 ± 1.3[b]	85.7 ± 4.1
8	94.2 ± 0.8	77.2 ± 2.6
24	94.9 ± 2.4	87.6 ± 1.3
48	93.7 ± 1.2	81.0 ± 4.6
72	95.5 ± 1.9	81.9 ± 5.6
	----------Depuration----------	
76	94.4 ± 0.6	76.6 ± 7.6
80	94.2 ± 2.7	65.9 ± 8.1
96	95.4 ± 1.7	70.6 ± 9.5

[a] Organisms were depurated after 72 hours of exposure.
[b] All values mean ± S.D., N = 3.

tissue as acetone-extractable throughout the entire 96 hours of exposure. However, results indicate that the percent nonextractable ^{14}C activity was greater for the phenol-plus-WSF exposure (intercepts were significantly different at $\alpha < 0.01$) than for the phenol-only exposure. The acetone-extractable ^{14}C activity decreased from 86% at 4 hours after initiation of exposure to 71% at 96 hours.

Water concentrations remained stable throughout the 72-hour exposure period for both aniline-only and aniline-plus-WSF exposures with trout. Mean ^{14}C activity in the aniline only exposure was 10,300 dpm/mL; it was 10,800 dpm/mL for the aniline-plus-WSF exposure.

A comparison of the results of the total uptake of ^{14}C activity by rainbow trout exposed to ^{14}C aniline and ^{14}C aniline in the presence of the WSF is shown in Table 2. In the aniline-only exposure, total ^{14}C activity in whole fish tissue increased from a mean value of 56,000 dpm/g at 4 hours after initiation of the exposure to 203,000 dpm/g at 72 hours. Body burdens of trout placed in clean water decreased to 50% of the high mean ^{14}C activity after 24 hours of depuration. In the aniline-plus-WSF exposure, ^{14}C activity ranged from a mean value of 57,000 dpm/g at 4 hours after initiation of exposure to 203,000 dpm/g at 72 hours. The body burdens of fish placed in clean water after 72 hours decreased to 45% of the high mean ^{14}C activity observed at 96 hours.

Based on concentrations of ^{14}C in whole body fish and exposure water, ^{14}C bioconcentration factors were estimated for accumulation of ^{14}C activity in rainbow trout exposed to water containing aniline only and aniline plus WSF. The bioconcentration factor was estimated at 20 for aniline-only exposures and 19 for aniline-plus-WSF exposures.

In the aniline-only exposure, from 70% to 89% of the total ^{14}C activity was recovered as acetone-extractable activity during the course of the experiment (Table 3). The amount of acetone-extractable activity

TABLE 2. Total ^{14}C Activity (Extractable Plus Non-Extractable) in Rainbow Trout Exposed to ^{14}C Aniline and ^{14}C Aniline Plus Water-Soluble Fraction (WSF).

Exposure Time (hr)	Total ^{14}C Activity (dpm/g wet weight x 10^2)	
	^{14}C Aniline Only	^{14}C Aniline + WSF
	----------Uptake----------	
4	559 ± 25[(a)]	573 ± 24
8	785 ± 48	739 ± 8
24	1,333 ± 62	1,211 ± 89
48	1,622 ± 60	1,556 ± 78
72	2,032 ± 120	2,029 ± 169
	----------Depuration----------	
76	1,569 ± 178	1,187 ± 82
80	1,845 ± 344	1,502 ± 318
96	1,025 ± 41	904 ± 92

[(a)] All values mean ± S.E., N = 3.

decreased with time of exposure. Results for the aniline-plus-WSF exposure were similar to the aniline-only exposure at all time periods. The general linear test indicated no significant difference between slope or intercept of the two regression lines ($\alpha > 0.10$).

Mean concentrations of water in the phenol-only exposures with daphnids declined slightly from 115,300 dpm/ml at 0 hour to 105,200 dpm/ml at 24 hours. A similar decline was observed in the phenol-plus-WSF exposures with daphnids; maximum concentrations of 116,500 dpm/mL were observed at 2 hours and the lowest concentration of 102,200 dpm was noted after 8 hours of exposure. Small amounts of radioactivity were detected during depuration. Mean ^{14}C concentrations in the water, at 48

TABLE 3. Comparison of Distribution of Acetone-Extractable ^{14}C Activity in Rainbow Trout Exposed to Treatments of ^{14}C Aniline and ^{14}C Aniline Plus Coal Liquid Water-Soluble Fraction (WSF).[a]

Exposure Time (hr)	% Extractable ^{14}C Activity	
	^{14}C Aniline Only	^{14}C Aniline + WSF N = 3
	----------Uptake----------	
4	89.0 ± 1.2[b]	89.4 ± 1.6
8	87.1 ± 0.6	85.2 ± 2.1
24	86.8 ± 2.4	82.3 ± 2.8
48	82.7 ± 1.6	80.6 ± 2.5
72	77.8 ± 3.8	74.5 ± 7.2
	----------Depuration----------	
76	70.8 ± 1.8	72.1 ± 3.2
80	73.7 ± 2.3	73.4 ± 2.8
96	69.9 ± 3.6	66.4 ± 4.1

[a] Organisms were depurated after 72 hours of exposure.
[b] All values mean ± S.D., N = 3.

hours, were near 200 dpm/mL for both treatments and may have resulted from excretion of absorbed phenol by the daphnids.

Mean ^{14}C activity in daphnids for the phenol-only treatment increased rapidly during the 24-hour uptake period and was greater for the phenol-plus-WSF treatment at all time intervals after 1 hour (Figure 2). 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 4). Significant differences ($\alpha \leq 0.001$) were detected in the uptake rates of ^{14}C between the single-compound and compound-plus-WSF-exposures.

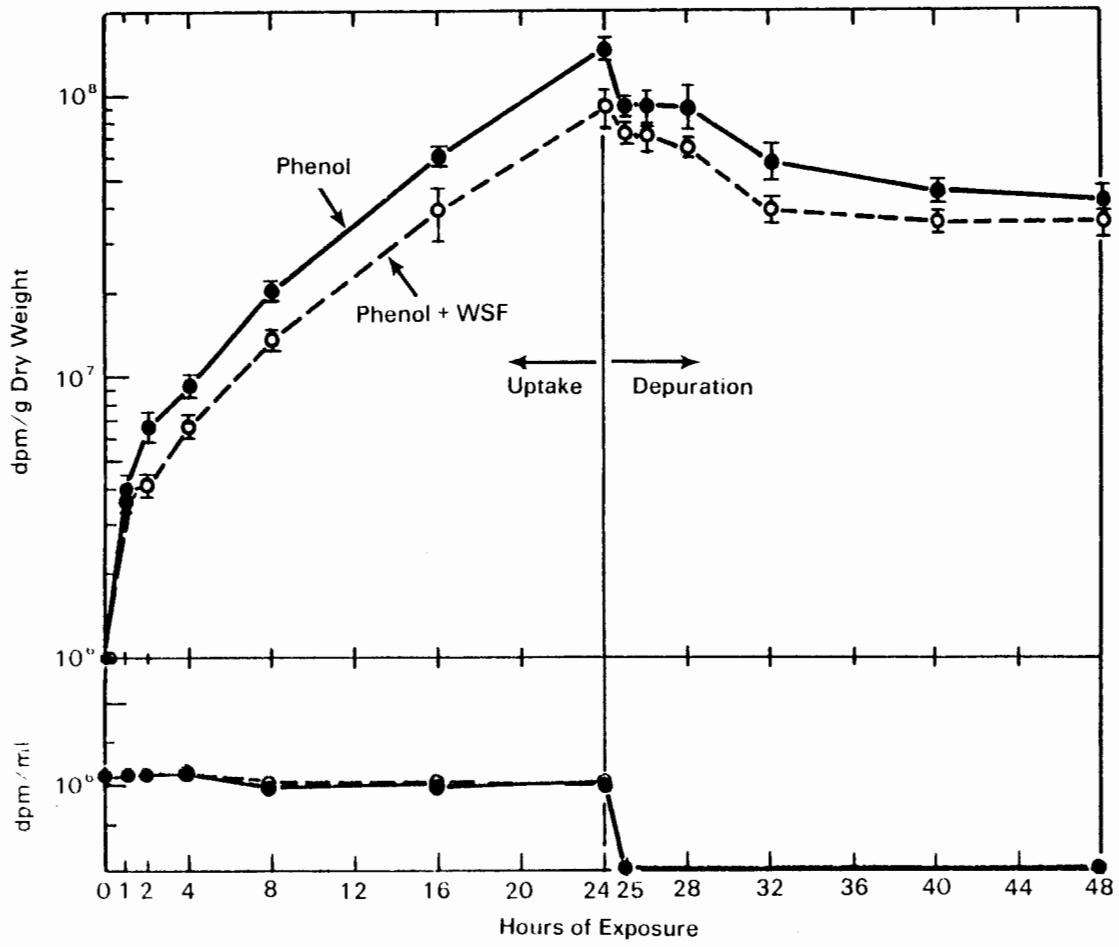

FIGURE 2. Comparison of ^{14}C Activity in Daphnids Exposed to Phenol Only and to Phenol in the Presence of a Coal Liquid Water-Soluble Fraction (WSF). Values given as mean ± S.E, N = 4.

However, no significant difference was found for the depuration rate. Estimated half-lives for the phenol-only and phenol-plus-WSF treatments were 8.0 hours and 12.1 hours, respectively.

Mean concentrations of water in the aniline-only exposures with daphnids declined from a maximum of 150,000 dpm/mL after 1 hour of exposure

TABLE 4. Estimates of Uptake and Depuration Rates and Bioconcentration Factor (BCF) for Daphnids.

Compound	Uptake Rate (K_1)[a]	Elimination Rate (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 rates estimated from the equation $C_d = (K_1/K_2)C_w(1 - e^{-K_2 t})$, where K_1 is the uptake rate, depuration rate, C_w is the dpm of ^{14}C labeled compound in the water at steady state, C_d is compound concentration in Daphnia, and t is time.

[b] Elimination rates estimated from the equation $C_d = H1^{e-K_2 t}$, where K_1 is the dpm in Daphnia at time ϕ.

[c] All values are mean ± 1 standard deviation.

to 139,200 dpm/mL after 24 hours. Concentrations in the aniline-plus--WSF treatment were similar; mean ^{14}C activity ranged from 144,500 dpm/mL at 1 hour to 135,700 dpm/mL after 8 hours of exposure. Negligible ^{14}C activity was detected in water during depuration; concentrations at 48 hours were near 50 dpm/mL.

Total ^{14}C activity in daphnids was similar at each of the sample intervals for both treatments (Table 5). Based on concentrations of ^{14}C in daphnids and exposure water, ^{14}C bioconcentration factors at peak

TABLE 5. Comparison of ^{14}C Activity in Daphnids Within Phenol and Phenol-Plus-WSF Exposures During a 24-Hour Uptake and a 24-Hour Depuration Phase.

Exposure Time (hr)	Total ^{14}C Activity (dpm/g x 10^4)	
	Aniline Only	Aniline + WSF
0	(22 ± 1)[a][b]	(22 ± 1)[a]
1	263 ± 19	248 ± 21
2	416 ± 78	285 ± 82
4	608 ± 79	410 ± 45
8	585 ± 51	736 ± 89
16	906 ± 138	1,022 ± 106
24	1,016 ± 119	1,058 ± 166
25	864 ± 120	980 ± 147
26	1,030 ± 139	895 ± 154
28	933 ± 193	889 ± 113
32	780 ± 152	577 ± 95
40	689 ± 146	834 ± 170
48	527 ± 72	545 ± 122

[a] Values in disintegrations per minute (dpm).
[b] Values are mean ± S.E.

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 using only the uptake data, but are considerably less than estimates that used depuration rates from the depuration phase data (Table 4). There was no significant difference ($\alpha > 0.05$) in either the uptake or depuration rates between the two treatments. Estimated ^{14}C half-lives were 26.5 hours for both the aniline-only and aniline-plus-WSF exposures.

DISCUSSION

These studies demonstrated that differences may exist in uptake, depuration, and storage when a single compound is presented to an organism, as opposed to the same compound presented within a complex chemical mixture. The differences observed in the studies 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. Although data in whole fish were variable, approximately 35% less radioactivity was accumulated by fish, after 72 hours, when coal liquid water-soluble components were present. Uptake rates of ^{14}C phenol by daphnids were also lower in the presence of the coal liquid WSF, when compared to the single-compound exposure. This suggests that other lipophilic components competed with phenol for absorption into tissue.

Since metabolites were not characterized, relative contribution of percent compound to total ^{14}C activity is unknown. However, it is expected that a relatively high percentage of the test compounds were metabolized. In studies with the phytoplankter Scenedesmus quadricanuda (Hardy, Dauble and Felice 1984) significant quantities of aniline and phenol were biotransformed to metabolites in 24 hours. Call, Brooke and Lu (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 (Melancon and Lech 1978).

Our studies also suggest that the presence of a complex organic mixture may influence metabolic and storage processes. Significantly smaller portions of absorbed ^{14}C activity could be extracted from fish exposed to phenol when soluble coal liquid components were present. In

long-term (e.g., 28-day) exposures of fathead minnow, Call, Brooke and Lu (1980) found that levels of acetone-unextractable ^{14}C were somewhat concentration-dependent and ranged from 78.5% to 89.1% of the total radioactivity. A trend towards increasing amounts of unextractable radioactivity was evident only for our phenol-plus-WSF treatment. The values reported in this study are consistent with those reported in other short-term exposure studies. For example, Southworth, Keffer and Beauchamp (1981) reported that 10% of the ^{14}C in fathead minnow was unextractable following 108 hours of exposure to benz(a)acridine.

Although equal concentrations of phenol were present in both mixtures, increased concentrations of other chemicals in the WSF mixture may have induced enzyme systems in the fish. Thus, observed differences in unextractable residues could be due to selective binding of phenol or metabolites to tissue storage sites. For daphnids, depuration rates were similar for all cases, suggesting that mechanisms of elimination were unaffected by prior exposure to the complex mixture.

In contrast to phenol, presence of coal liquid water solubles did not significantly influence either the uptake or elimination of ^{14}C aniline by either daphnids or rainbow trout. Aniline uptake, depuration and relative quantities of acetone extractables in trout tissue were essentially the same in the presence and absence of the WSF. This would suggest that the sites for absorption of aniline to trout tissue are limited and of a different chemistry than those for phenol.

Differences in potential for bioaccumulation of phenol in complex mixtures are not consistent with estimates of BCF as determined by measured octanol/water coefficient values (Table 6). BCF estimates of phenol, based on $\log_{10}$ Kow 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, Defoe and Bergstedt (1979) who, in

TABLE 6. Comparison of Octanol/Water Partition Coefficients Derived for Aniline and Phenol as Single Compounds and in Aqueous Extracts of an SRC II Liquid.

Compound	Log_{10} Kow[a]	Log_{10} Kow in WSF[b]
Phenol	1.46	1.43
Aniline	0.90, 0.98	0.96

[a] From Leo, Hansch and Elkins (1971).
[b] From Thomas (1984)

exposing fathead minnow to individual and mixed solutions of p,p'DDE and heptachlorepoxide, obtained similar BCFs for both. However, Veith, Defoe and Bergstedt postulated that uptake of chemicals in mixtures would only be independent of other chemicals provided that metabolism of the organism was not significantly altered.

These experiments, necessary precursors to more detailed studies, nonetheless suggest that behavior of compounds in complex mixtures may not always be predicted based on single-compound exposures. The entire spectrum of environmental chemicals can influence uptake and storage mechanisms within an organism. Interactions among individual components within complex mixtures may also be expected to alter their retention, metabolism and excretion. Future studies, therefore, should examine a range of compound classes in addition to determining the rate of metabolism and excretion of parent compounds in complex mixtures.

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