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Journal Article WWRC-84-16

In

Environmental Toxicology
and Chemistry

Volume 4
1985

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Environmental Toxicology and Chemistry Vol. 4, pp. 549-558, 1985
Printed in the USA. Pergamon Press Ltd.

0730-7268/85 $3.00 + .00

ANTHRACENE BIOCONCENTRATION IN RAINBOW TROUT DURING SINGLE-COMPOUND AND COMPLEX-MIXTURE EXPOSURES

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(*Received* 18 *June* 1984; *Accepted* 7 *December* 1984)

Abstract—Rainbow trout were exposed for 72 h to [^{14}C]anthracene alone and [^{14}C]anthracene in an oil shale retort water. Tissue levels of [^{14}C]anthracene were analyzed at 24, 48 and 72 h to determine non-steady-state bioconcentration factors (BCFs), and uptake and depuration rates were calculated from anthracene disappearance in exposure waters and metabolite appearance in depuration waters. Uptake rates (14.6 to 16.9 h^{-1}) were similar to previously reported values; however, depuration rates (0.00158 to 0.00188 h^{-1}) were very low. Consequently, measured non-steady-state BCFs after 72 h (9,000 to 9,200) for anthracene were higher than expected, probably because fish were not fed and had low excretion rates. Measured and estimated anthracene BCFs were lower in retort water exposures than in single-compound exposures because of slower uptake and faster depuration. We attribute slower uptake during retort water exposures to either decreased bioavailability of anthracene or rate-limiting transport of contaminants from uptake sites to storage and processing sites. Induction of mixed-function oxidases may have increased depuration from fish exposed to retort water. We conclude that bioconcentration of individual compounds from complex chemical mixtures may be difficult to predict based on single-compound kinetics.

Keywords—[^{14}C]Anthracene Oil shale retort water Rainbow trout Uptake Depuration

INTRODUCTION

Complex chemical mixtures enter aquatic environments from many sources, including agricultural and municipal runoff, industrial effluents, treatment plant discharges and atmospheric depositions. Furthermore, numerous nonanthropogenic chemicals occur naturally in surface waters. Generally, interactions between a chemical and other contaminants or naturally occurring compounds are ignored or assumed to be insignificant in environmental fate studies and predictive models. But evidence is slowly accumulating that chemical and physical interactions within complex mixtures may alter bioavailability, and hence alter the fate and effects of individual contaminants.

For example, results of the relatively few bioconcentration studies conducted to date using chemical mixtures suggest that bioconcentration of organic compounds is usually decreased by adsorption to humic acids [1-3] and by interactions with other contaminants [4-6]. Hence, the bioconcentration factor (BCF) of an organic chemical predicted from classical, single-compound kinetic models [7,8] may differ considerably from the realized BCF following exposure to a complex chemical mixture.

We report herein the BCF differences in rainbow trout for a polynuclear aromatic hydrocarbon, anthracene, during single-compound and complex-mixture exposures. We argue that decreased uptake and increased depuration accounted for the lower bioconcentration of anthracene from oil shale retort water. We also propose a new method for estimating uptake and depuration rates in static, non-steady-state exposure systems.

MATERIALS AND METHODS

Experimental fish

Sexually immature rainbow trout (*Salmo gairdneri*) were obtained from the Wyoming Game and Fish Department and maintained at the University of Wyoming in flowing, aerated laboratory water for 2 weeks prior to experimentation. We fed trout chow (Silver Cup, Murray, UT) *ad libitum* to the fish until 2 d before the contaminant exposures were begun. Water temperatures ranged from 11 to 13°C during the preexperimental holding period.

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Table 1. Chemical characteristics of laboratory dilution water

Parameter	Value
Alkalinity, total (as $CaCO_3$)	160
Conductivity (μS/cm at 25°C)	820
Hardness (as $CaCO_3$)	470
Oxygen, dissolved	6.5
pH (units)	8.3

Values expressed as mg/L, unless otherwise noted.

Table 2. Chemical characteristics of Oxy-6 oil shale retort water[a]

Parameter	Value
Alkalinity, total (as $CaCO_3$)	6,700
Carbon, total organic	2,900
Chemical oxygen demand	7,900
Conductivity (μS/cm at 25°C)	12,800
Hardness (as $CaCO_3$)	10
Nitrogen	
Ammonia (as N)	800
Kjeldahl (as N)	1,000
pH (units)	8.9
Solids, total dissolved	10,200

Values expressed as mg/L, unless otherwise noted.
[a]Data from Marcus and Bergman [11].

Photoperiod was maintained at 16 h light:8 h dark during both the holding period and the subsequent uptake and depuration studies.

Experimental design

The bioconcentration experiment consisted of a 72-h uptake phase immediately followed by a 144-h depuration phase. Fish were tested in 15-liter, static-renewal aquaria, with two replicate aquaria per exposure treatment and a single control aquarium. Water was replaced every 24 h during the uptake phase and every 8 h during the depuration phase. We aerated all aquaria to maintain adequate dissolved oxygen concentrations, and controlled temperatures at 12 ± 0.5°C by placing the aquaria in flow-through cooling baths. Fish were not fed during the experiment. All aquaria were covered with dark lids to reduce anthracene photodecomposition; however, light still penetrated the transparent glass walls of the aquaria so fish could perceive the diel photoperiod.

A mixture of well water and dechlorinated Laramie city water was used in control and depuration aquaria and for diluting single-compound and complex-mixture exposure solutions. The characteristics of this dilution water are given in Table 1. During uptake and depuration phases we frequently measured dissolved oxygen and total ammonia concentrations using a Winkler-calibrated YSI Model 54A oxygen meter and an Orion Model 407A Ionalyzer equipped with an ammonia electrode. Other water quality parameters, including pH, alkalinity, conductivity and hardness, were analyzed using standard methods [9].

Uptake

For the uptake phase, fish were exposed to (a) dilution water only, (b) anthracene in dilution water (unlabeled anthracene, 98%, Aldrich Chemical Co., and [9-^{14}C]anthracene, 3.3 mCi/mM, 98%, California Bionuclear Corp.), or (c) anthracene [cold and radiolabeled, as in (b)] and a complex chemical mixture in dilution water. The complex mixture was an oil shale retort water collected from the Occidental Oil Shale Company modified *in situ* retorting facility at Logan Wash, Colorado, and was designated Oxy-6.

Like most oil shale retort waters, Oxy-6 is highly alkaline and contains high concentrations of inorganic and organic solutes (Table 2). A reversed-phase, high performance liquid chromatography (HPLC) gradient fingerprint illustrates the variety of organic chemical classes present, ranging from relatively polar carboxylic acids, phenolics and aliphatic amides to nonpolar monocyclic and polycyclic aromatic hydrocarbons (Fig. 1; see also ref. 10). Based on toxicity tests conducted previously in our laboratory, Oxy-6 retort water is highly toxic to fish. For rainbow trout, the 96-h LC50 was 0.35%, and the no observed effect concentration (NOEC), as determined in a 60-d embryo-larval growth test, was 0.0045% [11].

To start the experiment, unlabeled anthracene was added to single-compound exposure aquaria 4 h before 15 fish were transferred to them, and [^{14}C]anthracene was added 3 h after unlabeled anthracene. In complex-mixture exposures, Oxy-6 retort water was added at the same time as the unlabeled anthracene. Analyzed anthracene concentrations in all exposure aquaria were approximately 50 μg/L; the nominal retort water concentration dosed in complex-mixture exposures was 0.0045% (the NOEC for rainbow trout). In a preliminary experiment we established that during an 18-h period there was no detectable biodegradation or volatilization of anthracene in control water containing no fish.

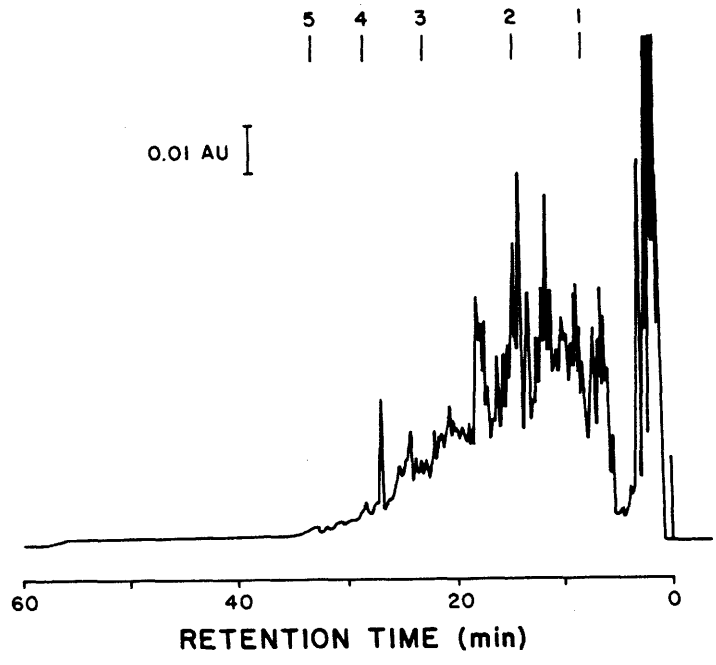

Fig. 1. Reversed-phase HPLC gradient fingerprint of Oxy-6 retort water. Chromatographic conditions were as follows: 30-cm μ-C_{18} Bondapak column; 50 μl injection; 60-min linear gradient programmed from 100% H_2O to 100% CH_3CN (acetonitrile) at 2.0 ml/min; 254 nm UV detector. Retention times of five representative compounds are indicated above the chromatogram: 1, resorcinol; 2, phenol; 3, benzene; 4, toluene; 5, naphthalene.

Water was sampled at 0, 1, 2, 3, 4, 6, 10, 16 and 24 h during every 24-h replacement interval of the uptake phase. At 0, 24, 48 and 72 h, we removed two fish per replicate for tissue residue analyses.

Depuration

Immediately after the end of the 72-h uptake phase, fish were transferred to aquaria containing clean laboratory water so that clearance of anthracene and metabolites could be monitored for 144 h. We sampled water at the end of each 8-h replacement period, and removed two fish per replicate every 48 h.

Water analyses

Water samples were analyzed for radiolabeled anthracene and metabolites using liquid scintillation counting (LSC) and HPLC techniques. First, total ^{14}C activity was determined on a Beckman LS-100C liquid scintillation spectrophotometer after dissolving 1-ml aliquots of each sample in a toluene/Triton X-100/POP/POPOP cocktail [12]. We also fractionated the original water samples on a Waters Associates Model 204 high performance liquid chromatograph equipped with a 30-cm, μ-C_{18} reversed-phase column and a Model 440 UV absorbance detector. Samples were eluted isocratically in 70% CH_3CN/30% H_2O at 2.0 ml/min, and were monitored for anthracene at 254 nm on the UV detector. From each water sample, we collected the parent anthracene peak and divided the eluate prior to anthracene's retention time into three 90-s fractions. Then, these anthracene and preanthracene fractions were assayed for ^{14}C activity to determine metabolite distributions. Because organic compounds usually elute from reversed-phase HPLC columns in order of decreasing polarity, we assumed that all anthracene metabolites eluted prior to anthracene's retention time.

Fish analyses

After removal from uptake or depuration aquaria, we killed the fish by severing their spinal cords immediately posterior to the occipital region; then we rinsed them in well water and blotted them dry. We weighed the fish and either processed them immediately for residue analysis or froze them for later examination.

To extract anthracene and metabolites from fish, we used an exhaustive steam distillation (ESD) procedure originally described by Veith and Kiwus [13] and modified by Meyer et al. [14]. Briefly, we homogenized the fish and boiled each homogenate in 400 ml of 1% H_2SO_4 under reflux for 16 h. Condensed vapors passed through an extraction solvent reservoir in the condenser column before returning to the boiling flask, thus partitioning anthracene and its metabolites into the extraction solvent. In a previous study, we demonstrated that anthracene is extracted with 92% efficiency using an ESD solvent mixture of 75% *n*-butyl ether/20% cyclohexane/5% *n*-octanol [14].

This solvent mixture was optimized to extract a wide range of aromatic compound polarities, including anthracene and polar metabolites.

After ESD refluxing was completed, we analyzed all liquid phases (extraction solvent, extraction reservoir water layer and boiling flask liquid) for [^{14}C]anthracene and metabolites, using the same HPLC and LSC techniques described above for water samples. Mass balances were then computed for each fish. Additionally, 0.1-g subsamples of the original tissue homogenates were dissolved in 2 ml of NCS Tissue Solubilizer (Amersham Corp.) and assayed for total ^{14}C activity so that ESD mass balances could be verified.

BCFs and rate constants

Anthracene BCFs were calculated two ways. First, [^{14}C]anthracene levels determined in fish tissue by ESD extraction were divided by the geometric means of the [^{14}C)anthracene concentrations in exposure aquaria during the uptake phase. In the following discussion, we refer to these ratios as non–steady-state BCFs.

For the second BCF calculation, we assumed a single-compartment, first-order kinetic model of uptake and depuration, as proposed by Branson et al. [7]. According to this model, the rate of change in concentration of a chemical in a fish can be expressed as

$$\frac{dC_f}{dt} = k_u C_w - k_d C_f \quad (1)$$

where C_f is the concentration in fish, C_w is the concentration in water, k_u is the uptake rate constant, k_d is the depuration rate constant, and t is time. At steady state, Equation 1 rearranges to

$$\text{BCF} = \frac{C_f}{C_w} = \frac{k_u}{k_d} \quad (2)$$

Conventionally, if C_w is constant, then the uptake rate can be estimated from the equation for the tangent to the initial uptake curve:

$$C_f = C_0 + k_u C_w t \quad (3)$$

where C_0 is the concentration in fish at the beginning of the uptake phase [3]. During depuration, the first-order elimination rate can be expressed by

$$\ln C_f = \ln C_{f,0} - k_d t \quad (4)$$

where $C_{f,0}$ is the concentration in fish at the beginning of the depuration phase [3].

In static exposures such as we used in this experiment, however, C_w is not constant. Thus Equation 1 cannot be integrated to produce Equation 3. To circumvent this difficulty, we alternatively express the concentration derivative as

$$\frac{dC_f}{dt} = -\frac{M_w}{M_f}\frac{dC_w}{dt} \quad (5)$$

where M_f is the mass of fish (g) and M_w is the mass of exposure water (g) (note that for water this is equivalent to volume, expressed in milliliters). Substituting Equation 5 into Equation 1 and rearranging yields

$$\frac{dC_w}{dt} = \frac{M_f}{M_w}(k_d C_f - k_u C_w) \quad (6)$$

At the beginning of the uptake phase, $k_u C_w \gg k_d C_f$ (the same assumption used to derive Equation 3 from Equation 1). Thus, integrating Equation 6 produces

$$\ln C_w = \ln C_{w,0} - k_u \frac{M_f}{M_w} t \quad (7)$$

where $C_{w,0}$ is the initial concentration in exposure water. In this experiment, we estimated k_u from the least-squares regression slope of $\ln C_w$ versus t during the first 4 h of uptake, where C_w is the [^{14}C]anthracene activity in exposure waters (dpm ml^{-1}). To determine these [^{14}C]anthracene activities, we fractionated the total ^{14}C activity in exposure waters using the HPLC technique described above and then analyzed the anthracene fraction by LSC. Thus, uptake rate estimates did not include metabolites excreted into the exposure waters.

During the depuration phase, $k_u = 0$, and $C_f = C_{f,0} - C_w(M_w/M_f)$. Substituting these relations into Equation 6 yields

$$\frac{dC_w}{dt} = k_d\left(C_{f,0} - C_w\frac{M_w}{M_f}\right) \quad (8)$$

For slowly depurated compounds, $C_{f,0} \gg C_w \times (M_w/M_f)$ initially. Therefore, integrating Equation 8 produces

$$C_w = k_d C_{f,0}\frac{M_f}{M_w} t \quad (9)$$

In this experiment, we estimated k_d from the least-squares regression slope of $C_w(M_w/M_f)$ versus t during the first 24 h of depuration, where C_w is the ^{14}C activity in depuration water (dpm ml^{-1}) and $C_{f,0}$ is the initial [^{14}C]anthracene activity in the fish transferred to the depuration aquarium (dpm g^{-1}). During the clearance phase, we fractionated the ^{14}C activity in aquarium waters using the HPLC technique described. However, since no parent [^{14}C]anthracene could be detected, the total ^{14}C activity values used in depuration rate calculations represent clearance of anthracene metabolites only. Tissue extracts of fish were fractionated similarly and only [^{14}C]anthracene levels in fish tissue were used to calculate $C_{f,0}$.

Finally, we computed BCF values from the ratio k_u/k_d. In the following discussion, we refer to these values as estimated steady-state BCFs.

RESULTS

Average non-steady-state BCFs computed from rainbow trout tissue analyses after 24, 48 and 72 h of exposure were at least 25% lower for fish exposed to anthracene in Oxy-6 retort water than for fish exposed to anthracene alone (Fig. 2). At 72 h, the average BCFs were 571 in Oxy-6 retort water and 779 in anthracene-only exposures. Mean fish weights did not differ between treatments ($\alpha = 0.05$; Student's t test), and variances were homogeneous (Hartley's test). For all 75 experimental rainbow trout, mean weight and standard deviation were 10.0 ± 4.0 g.

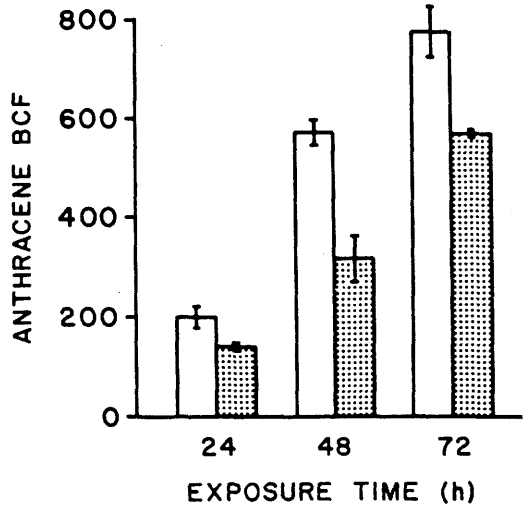

Fig. 2. Bioconcentration of [^{14}C]anthracene in rainbow trout during the 72-h uptake period for fish exposed to either a single compound (anthracene alone, open columns) or a complex chemical mixture (anthracene in Oxy-6 retort water, stippled columns). Ranges for bioconcentration factors (BCF) in replicate aquaria are indicated for each exposure system.

To estimate steady-state BCFs, we computed uptake and depuration rates based on aqueous concentrations of [^{14}C]anthracene and its metabolites. From initial slopes of $\ln C_w$ versus t (Figs. 3a and 4a), we calculated k_u to be 14.6 and 16.9 h^{-1} for replicate exposures to anthracene alone, and k_u to be 13.8 and 10.3 h^{-1} for replicate exposures to anthracene in Oxy-6 retort water (Table 3).

Table 3. Uptake and depuration rates and bioconcentration factors for anthracene in rainbow trout

	Anthracene alone		Anthracene in retort water	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Uptake				
M_f (g)	136	133	165	167
M_w (g)	1.5×10^4	1.5×10^4	1.5×10^4	1.5×10^4
$\ln C_w$ vs. t (h^{-1})[a]	−0.132	−0.150	−0.152	−0.115
k_u (h^{-1})	14.6	16.9	13.8	10.3
Depuration				
$C_{f,0}$ (dpm g^{-1})	1.37×10^5	1.16×10^5	0.94×10^5	0.75×10^5
$C_w \frac{M_w}{M_f}$ vs. t (dpm g^{-1} h^{-1})[b]	217	218	204	175
k_d (h^{-1})	1.58×10^{-3}	1.88×10^{-3}	2.16×10^{-3}	2.34×10^{-3}
Bioconcentration				
BCF	9,200	9,000	6,400	4,400

[a]Initial slope of $\ln C_w$ vs. t was based on water analyses during the first 4 h of exposure to [^{14}C]anthracene.
[b]Initial slope of $C_w(M_w/M_f)$ vs. t was based on water analyses during the first 24 h of depuration.

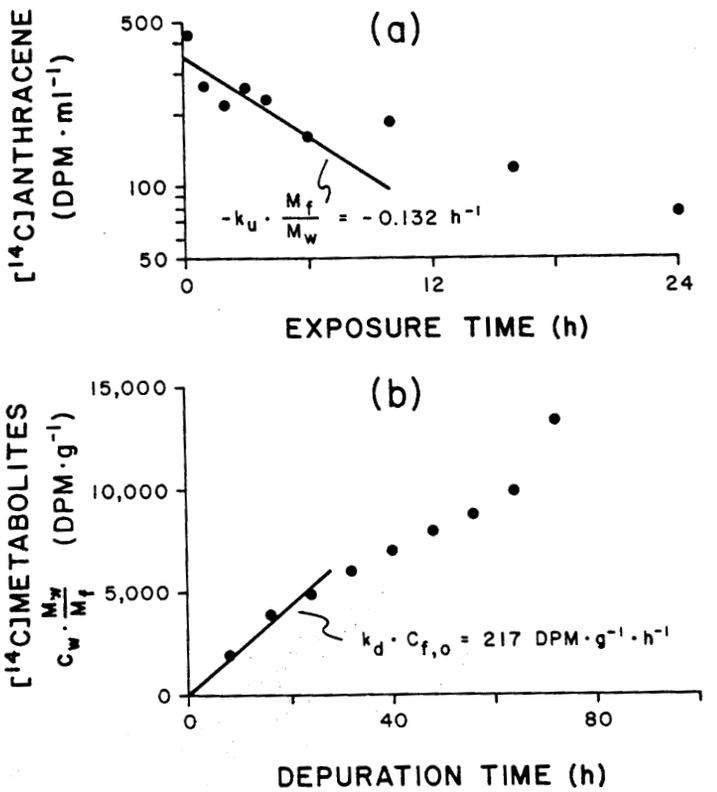

Fig. 3. Disappearance of [^{14}C]anthracene from exposure water **(a)** and appearance of [^{14}C]metabolites in depuration water **(b)** for one replicate of fish exposed to anthracene alone. See the text for equations.

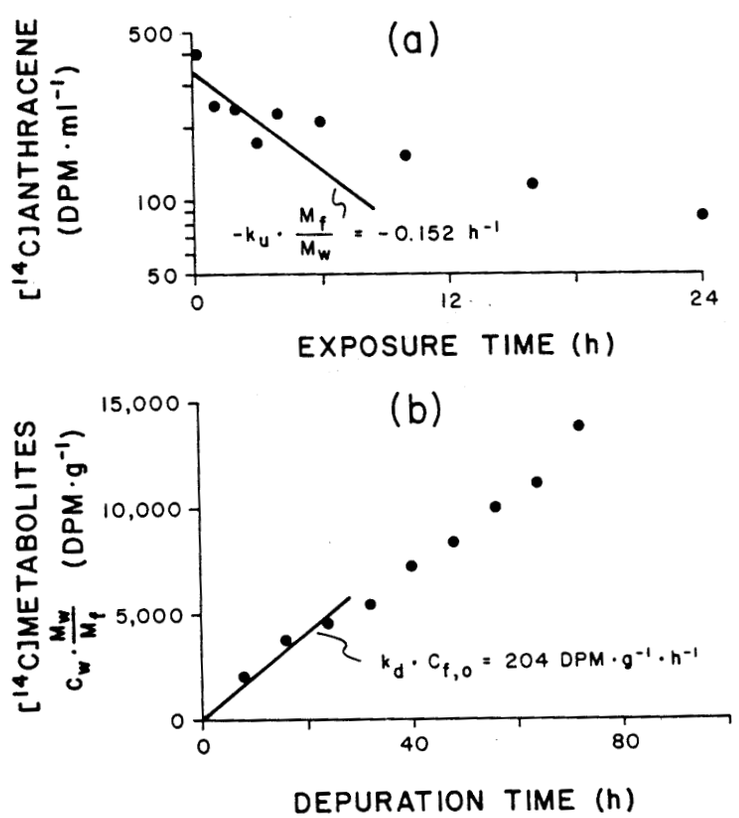

Fig. 4. Disappearance of [^{14}C]anthracene from exposure water **(a)** and appearance of [^{14}C]metabolites in depuration water **(b)** for one replicate of fish exposed to anthracene in Oxy-6 retort water. See the text for equations.

Depuration rates were based on concentrations in fish tissue after 72 h of exposure (Table 3) and on initial slopes of $C_w(M_w/M_f)$ versus t (Figs. 3b and 4b). These rates were $k_d = 0.00158$ and 0.00188 h^{-1} for replicate exposures to anthracene alone, and $k_d = 0.00216$ and 0.00234 h^{-1} for replicate exposures to anthracene in Oxy-6 retort water (Table 3). BCFs estimated from these uptake and depuration rates are 9,200 and 9,000 for anthracene alone, and 6,400 and 4,400 for anthracene in Oxy-6 retort water.

DISCUSSION

After 72 h, the non-steady-state anthracene BCF of 779 for rainbow trout at 12°C was already greater than the steady-state anthracene BCF of 675 estimated by Spacie et al. [3] for bluegill sunfish at 23 to 24°C. Of course, BCFs are difficult to estimate in static exposure systems, since the exposure concentration is not constant. We divided measured tissue levels of anthracene by the geometric mean water concentration, which was 160 dpm ml^{-1} for trout exposed to anthracene alone. Although the initial exposure concentration at the start of each 24-h replacement period was much higher (>400 dpm ml^{-1}), the [^{14}C]anthracene activity was less than 200 dpm ml^{-1} for 64 of the 72 h of exposure (Fig. 5). Therefore, we believe that the geometric mean concentration was representative of the exposure regime, and the relatively large non-steady-state BCFs were not artifacts of the calculation procedure.

To estimate steady-state BCFs, we have proposed a new method that relies on analyses of exposure and depuration water concentrations rather than tissue concentrations of the contaminant. As one advantage, the uptake and depuration experiments are conducted in static exposure systems, thus reducing expense and maintenance, as compared with flow-through exposures. Furthermore, the amount of contaminant used is minimized, thus helping to conserve radionuclides that are potentially expensive or in limited supply. In theory, no fish tissue analyses are needed to compute the uptake rate, and only one tissue analysis ($C_{f,0}$, Equation 9) is needed to compute a depuration rate. Even this tissue analysis can be circumvented if the exposure period is short and all of the contaminant that disappears from the exposure water is assumed to accumulate in the fish (i.e., depuration is still negligible). Then,

$$C_{f,0} = (C_{w,0} - C_{w,t})\frac{M_w}{M_f} \qquad (10)$$

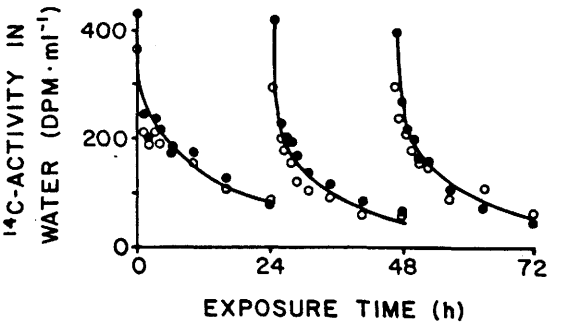

Fig. 5. Disappearance of [^{14}C]anthracene during the 72-h uptake period from exposure waters containing either a single compound (anthracene alone, filled circles) or a complex chemical mixture (anthracene in Oxy-6 retort water, open circles). Means of replicate aquaria are plotted for each exposure system.

where $C_{w,t}$ is the final concentration in exposure water, and $C_{f,0}$, $C_{w,0}$, M_w and M_f are as defined earlier. In effect, uptake and depuration rates can be estimated from water concentrations alone, thus saving considerable time and expense. However, analyzing fish tissue at the end of the uptake period would increase the reliability of calculations based solely on water concentrations.

Uptake rates of anthracene in rainbow trout at 12°C ($k_u = 14.6$ to 16.9 h^{-1}) computed by this method compared favorably with anthracene uptake rates reported by Spacie et al. [3] in bluegill sunfish at 23 to 24°C ($k_u = 36$ h^{-1}). Reduced uptake in our experiment was probably caused by species and temperature differences.

However, anthracene depuration rates in our study (0.00158 to 0.00188 h^{-1}) were less than one-tenth the value reported by Spacie et al. ($k_d = 0.040$ h^{-1}) [3]. Consequently, our estimated anthracene BCF (9,000 to 9,200) was much larger than their experimentally estimated value of 675, and the BCF of 1,200 predicted from anthracene's octanol/water partition coefficient [3].

These lower depuration rates are not an artifact of calculations using Equation 9. Plots of $\ln C_f$ versus t illustrate the large decrease in elimination of ^{14}C activity from trout in our experiment as compared with data for sunfish reported by Spacie et al. [3] (Fig. 6). In fact, depuration rates based on the slope of that plot are very similar to calculations based on Equation 9. Therefore, there appear to have been real differences in depuration between the two studies.

As mentioned above in explanation of decreased uptake rates, species differences and lower temperature may also account for some of the decrease in depuration rate. Perhaps even more

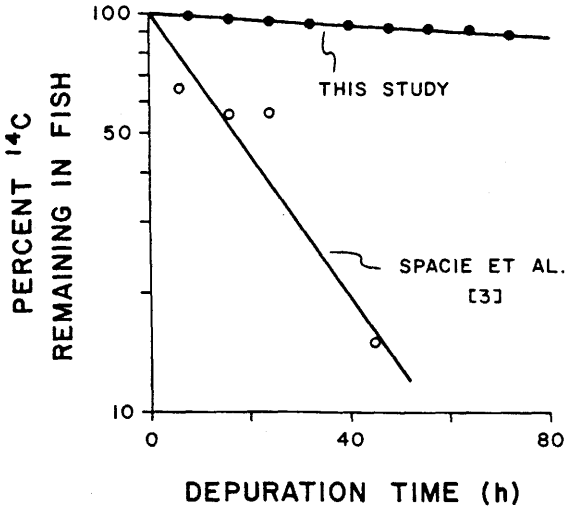

Fig. 6. Comparison of elimination of ^{14}C activity from rainbow trout in this study (filled circles) and from bluegill sunfish (open circles) as reported by Spacie et al. [3].

importantly, fish were not fed for 5 d prior to the start of the elimination phase in our experiment, whereas sunfish tested by Spacie et al. [3] were starved for only 4 h prior to the start of the elimination phase. Walton et al. [15] observed decreased activity of the hepatic aryl hydrocarbon hydroxylase (AHH) enzyme system in cunner starved for at least 5 weeks. However, we do not know if direct effects on rainbow trout AHH are possible after only 5 d of starvation.

Other effects of starvation were evident, though. Roubal et al. [16] and Lech and Bend [17] discuss the normal accumulation of high concentrations of metabolites in fish bile. Under starvation conditions, rainbow trout in our experiment retained unusually large volumes of bile in their gall bladders. At the same time, they defecated little and had little urine in their bladders. Therefore, we believe that metabolites were eliminated slowly because they accumulated in the gall bladder. Feeding would have stimulated release of bile, and metabolites would subsequently have been eliminated.

Our data do not allow us to differentiate between potential mechanisms for explaining the unusually low anthracene depuration rate. However, these results illustrate some of the problems associated with testing rapidly metabolized compounds that do not occur when testing persistent organics such as chlorinated hydrocarbons. Metabolites can accumulate in fish to higher levels than the parent compound if they are not eliminated rapidly [18]. Therefore, bioconcentration studies should be designed and interpreted with consideration of both metabolite dynamics and feeding rates.

Despite the potential dependence of depuration rates and bioconcentration on feeding regimes, it is apparent from measured non-steady-state BCFs that less anthracene accumulated in fish exposed to Oxy-6 retort water than in fish exposed to anthracene alone. These differences were caused by slower uptake of anthracene in the retort water and faster depuration of anthracene metabolites following this exposure.

Slower uptake rates may be explained by two hypotheses. First, anthracene bioavailability may have been lower in the retort water exposure because of physical-chemical binding to small, suspended particles or to colloidal aggregations of other organic chemicals. This effect would be similar to bioconcentration decreases reported by Leversee et al. [1] for anthracene in the presence of 2 mg/L humic acids, although Spacie et al. [3] did not observe decreased anthracene bioconcentration in the presence of 1 mg/L humic acids.

A second explanation for decreased uptake rates involves potential rate-limiting transport of anthracene from adsorption sites (e.g., gill) to tissue storage and processing sites (e.g., liver). In a mixture of organic chemicals, anthracene transport may be reduced by competition with other organics for carriers in the blood (e.g., erythrocytes and nonspecific binding proteins). A similar mechanism has been suggested for transport of carcinogens in mammalian blood [19]. However, we currently cannot reject either of these hypotheses to explain decreased uptake in the presence of Oxy-6 retort water.

Higher anthracene depuration rates in fish exposed to retort water may have been caused by induction of the mixed-function oxidase (MFO) enzyme system. For example, Payne and Penrose [20] reported induction of hepatic AHH activity in capelin and brown trout exposed to crude oil for 7 to 8 d and 15 to 16 d, respectively. Gruger et al. [21] also reported hepatic AHH induction after 6 d in coho salmon exposed to a water-soluble fraction of crude oil. In both studies, induction may have occurred before fish were first sampled. Finally, Walton et al. [15] showed that increases in hepatic AHH activity in cunner lagged only 1 to 3 d behind increases in exposure concentrations of crude oil. Although such rapid induction periods have not yet been demonstrated for rainbow trout, organic

chemicals in retort water may have induced the MFO system to metabolize anthracene faster and thus increase the depuration rate after only 3 d of exposure in our experiment.

Alternatively, decreased bioconcentration of anthracene in the presence of Oxy-6 retort water may be similar to the cosolvent effect that has been reported in some single-compound bioconcentration studies [5]. However, the calculated total organic carbon concentration at the Oxy-6 retort water NOEC exposure level used in this experiment was only approximately 0.13 mg/L. That organic carbon concentration was approximately 100 to 10,000 times less than concentrations of cosolvents, such as acetone, that are used to maintain lipophilic chemicals in solution for single-compound bioconcentration studies. Therefore, we do not believe that enough organic carbon was present in the diluted Oxy-6 retort water exposures for it to have behaved as a cosolvent.

In summary, measured non-steady-state BCFs and estimated steady-state BCFs demonstrated lower bioconcentration of anthracene in the presence of Oxy-6 retort water. We believe that accumulation of contaminants in fish exposed to complex chemical mixtures may in general be difficult to predict accurately from single-compound bioconcentration studies. In this short-term experiment, both uptake and depuration rates differed between single-compound and complex-mixture exposures. Results of our study and other published studies [4–6] suggest that single-compound bioconcentration experiments may provide conservative estimates of BCFs in complex mixtures (i.e., single-compound BCFs may be higher than BCFs that occur in complex mixtures). However, inhibition of degradation pathways by other chemicals present in a mixture (e.g., metals) would also be possible, thus leading to an underestimation of BCFs in complex-mixture studies. Longer studies are needed to test these possibilities and to investigate whether uptake and depuration rates can be altered to even greater extents by induction or inhibition of other biochemical systems in fish. Furthermore, a variety of complex effluents and defined chemical mixtures will have to be tested to better understand the environmental fates of complex chemical mixtures [4,6,14,22].

Acknowledgement—This research was supported by Cooperative Agreement No. CR808671 between the U.S. Environmental Protection Agency and the University of Wyoming, and by an NIEHS Postdoctoral Fellowship to the University of Cincinnati Medical Center under NIH Grant No. ES-07073. Additionally, J. S. Meyer was supported by the University of Wyoming Water Research Center during preparation of the manuscript. The Laramie Energy Technology Center of the U.S. Department of Energy kindly supplied the Oxy-6 retort water. Judy Bauer and Tom LaPoint reviewed an early draft of the manuscript and Bryan Steadman provided valuable comments throughout the study and manuscript preparation.

REFERENCES

1. **Leversee, G.J., P.F. Landrum, J.P. Giesy** and **T. Fannin.** 1983. Humic acids reduce bioaccumulation of some polycyclic aromatic hydrocarbons. *Can. J. Fish. Aquat. Sci.* **40** (Suppl. 2): 63–69.
2. **McCarthy, J.F.** 1983. Role of particulate organic matter in decreasing accumulation of polynuclear aromatic hydrocarbons by *Daphnia magna. Arch. Environ. Contam. Toxicol.* **12**:559–568.
3. **Spacie, A., P.F. Landrum** and **G.J. Leversee.** 1983. Uptake, depuration, and biotransformation of anthracene and benzo(a)pyrene in bluegill sunfish. *Ecotoxicol. Environ. Safety* 7:330–341.
4. **Bergman, H.L.** and **J.S. Meyer.** 1982. Complex effluent fate modeling. In K.L. Dickson, A.W. Maki and J. Cairns, Jr., eds., *Modeling the Fate of Chemicals in the Aquatic Environment.* Ann Arbor Science Publishers, Ann Arbor, MI, pp. 247–267.
5. **Landrum, P.F.** 1982. The effect of co-contaminants on the bioavailability of polycyclic aromatic hydrocarbons to *Pontoporeia hoyi.* In M.W. Cooke and A.J. Dennis, eds., *Polynuclear Aromatic Hydrocarbons. Seventh International Symposium on Formation, Metabolism and Measurement.* Battelle Press, Columbus, OH, pp. 731–743.
6. **Linder, G., H.L. Bergman** and **J.S. Meyer.** 1984. Constituent bioconcentration in rainbow trout exposed to a complex chemical mixture. *Bull. Environ. Contam. Toxicol.* **33**:330–338.
7. **Branson, D.R., G.E. Blau, H.C. Alexander** and **W.B. Neely.** 1975. Bioconcentration of 2,2′,4,4′-tetrachlorobiphenyl in rainbow trout as measured by an accelerated test. *Trans. Am. Fish. Soc.* **104**:785–792.
8. **Bishop, W.E.** and **A.W. Maki.** 1980. A critical comparison of two bioconcentration test methods. In J. Cairns, Jr., K.L. Dickson and A.W. Maki, eds., *Estimating the Hazard of Chemical Substances to Aquatic Life.* ASTM STP 657. American Society for Testing and Materials, Philadelphia, PA, pp. 35–54.
9. **American Public Health Association, American Water Works Association and Water Pollution Control Federation.** 1975. *Standard Methods for the Examination of Water and Wastewater,* 14th ed. Washington, DC.
10. **Leenheer, J.A., T.I. Noyes** and **H.A. Stuber.** 1982. Determination of polar organic solutes in oil-shale retort water. *Environ. Sci. Technol.* **16**:714–723.
11. **Marcus, M.D.** and **H.L. Bergman.** 1980. Effects of aqueous effluents from in situ fossil fuel processing technologies on aquatic systems. Annual Progress Report: 1 January–31 December 1979. U.S. DOE Report No. DOE/LETC/10058-T1. U.S. Department of Energy, Laramie, WY.

12. **Linder, G.** and **H.L. Bergman.** 1984. Periodic depuration of anthracene metabolites by rainbow trout. *Trans. Am. Fish. Soc.* **113**:513–520.
13. **Veith, G.D.** and **L.M. Kiwus.** 1977. An exhaustive steam-distillation and solvent-extraction unit for pesticides and industrial chemicals. *Bull. Environ. Contam. Toxicol.* **17**:631–636.
14. **Meyer, J.S., G.L. Linder** and **H.L. Bergman.** 1981. An approach for bioaccumulation studies of complex organic effluents: Extraction of representative organics from rainbow trout and water. In *Abstracts*, Third Annual Meeting, Society of Environmental Toxicology and Chemistry, 1981 November 22–24, Arlington, VA.
15. **Walton, D.G., W.R. Penrose** and **J.M. Green.** 1978. The petroleum inducible mixed function oxidase of cunner: Some characteristics relevant to hydrocarbon monitoring. *J. Fish. Res. Board Can.* **35**:1547–1552.
16. **Roubal, W.T., T.K. Collier** and **D.C. Malins.** 1977. Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon (*Oncorhynchus kisutch*). *Arch. Environ. Contam. Toxicol.* **5**:513–529.
17. **Lech, J.J.** and **J.R. Bend.** 1980. Relationships between biotransformation and the toxicity and fate of xenobiotic chemicals in fish. *Environ. Health Perspect.* **34**:115–131.
18. **Southworth, G.R., C.C. Keffer** and **J.J. Beauchamp.** 1980. Potential and realized bioconcentration. A comparison of observed and predicted bioconcentration of azaarenes in fathead minnow (*Pimephales promelas*). *Environ. Sci. Technol.* **14**:1529–1531.
19. **Capel, I.D., H.M. Dorrell, M. Jenner** and **D.C. Williams.** 1979. The distribution of some carcinogens in blood. *IRCS Medical Science* **7**:492.
20. **Payne, J.F.** and **W.R. Penrose.** 1975. Induction of arylhydrocarbon (benzo(a)pyrene) hydroxylase in fish by petroleum. *Bull. Environ. Contam. Toxicol.* **14**:112–116.
21. **Gruger, E.H., Jr., M.M. Wekell, P.T. Numoto** and **D.R. Craddock.** 1977. Induction of hepatic aryl hydrocarbon hydroxylase in salmon exposed to petroleum dissolved in seawater and to petroleum and polychlorinated biphenyls, separate and together, in food. *Bull. Environ. Contam. Toxicol.* **17**:512-520.
22. **Gruger, E.H., Jr., J.V. Schell, P.S. Fraser, D.W. Brown** and **D.C. Malins.** 1981. Metabolism of 2,6-dimethylnaphthalene in starry flounder (*Platichthys stellatus*) exposed to naphthalene and p-cresol. *Aquat. Toxicol.* **1**:37–48.