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Anthracene Bioconcentration and Biotransformation in Chironomids: Effects of Temperature and Concentration

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ABSTRACT

Effects of temperature and anthracene concentration on uptake (K_u) *and depuration* (K_d) *rate constants and bioconcentration factor* (^{14}C-*BCF*) *were determined for larvae of the midge* Chironomus riparius. *At constant temperature* (25°C) *the uptake rate constant estimated from 10 h and 30 h exposure and by the initial rates methods increased with concentration between 1·7 and 30·5 µg litre*$^{-1}$. *At constant concentration* (22 µg litre^{-1}), *the uptake rate constant was maximum at 25°C and less at 16° and 30°C. The apparent increase in depuration rate constant with concentration during 30 h exposure was not confirmed in experiments in which contaminated animals depurated in uncontaminated paper towel. The* ^{14}C-*BCF did not change as a function of temperature or anthracene concentration. BCF based on anthracene concentration was minimum at 25°C when biotransformation rate was highest, and was more than an order of magnitude lower than* ^{14}C-*BCF.*

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INTRODUCTION

Regulatory agencies are faced with predicting the fates of large numbers of compounds which are introduced into aquatic systems. Mathematical simulation models may be useful for this purpose. However, few rate constants for uptake, depuration or biotransformation are available for use in mechanistic, predictive, simulation models. Also, the effect of environmental factors on conditional rate constants is unknown. If conditional rate constants for uptake, depuration and biotransformation are not independent of these parameters, a statistical relationship may be needed for prediction of these constants.

Two important factors that affect the dynamics of compounds within aquatic systems and organisms are temperature and concentration of chemical. One aspect of our study was to determine the relationship between polycyclic aromatic hydrocarbon (PAH) concentration and the uptake and depuration rate constants and bioconcentration factor (BCF) in midge larvae. A second aspect of this study was to determine the effects of temperature on uptake, depuration, biotransformation, and BCF.

Anthracene is a representative of the homologous series of polycyclic aromatic hydrocarbons (PAH) with relatively low volatility (Southworth, 1979), and intermediate water solubility (Leo, 1975).

MATERIALS AND METHODS

Midges

Chironomus riparius larvae were collected from a sewage outfall on Badfish Creek near Madison, Wisconsin. Midges were reared in paper towel substrate using a method similar to that described by Leversee *et al.* (1982) at the Savannah River Ecology Laboratory for over 20 generations at 25 °C. Animals were acclimatised to the temperature regime of the experiment for 2 to 3 days prior to exposure to anthracene. Each sample contained 5 midges. Sample weights ranged from $2{\cdot}2 \pm 0{\cdot}1$ mg ($n = 30$) to $3{\cdot}9 \pm 0{\cdot}1$ ($n = 30$). Dry weight was 12·5% of wet weight.

Water and anthracene

Well water (pH 7·1) was aerated for several days and filtered through 0·45 μm Millipore filters to remove particulates. Anthracene (3·3 mCi mmole^{-1}, 9-^{14}C) was obtained from California Bionuclear Corp. (Lot

No. 770824) and used without further purification. Radiochemical purity was determined by thin layer chromatography to be greater than 98 %. All preparative, analytical and experimental procedures were performed under gold fluorescent light ($\lambda \geq 500$ nm) to avoid photodegradation of anthracene.

Dosing and sampling

Accumulation flux was measured and uptake and depuration rate constants estimated at four concentrations (1·7, 8·7, 22·3 and 30·5 μg litre^{-1}) at 25 °C, and three temperatures (16, 25 and 30 °C) at 22 μg litre^{-1}. Uptake and depuration rate constants (K_u and K_d, respectively) were estimated from several kinds of data. K_u and K_d were estimated simultaneously during uptake experiments. K_d was also estimated in several ways from data from depuration experiments.

Uptake experiments were conducted in glass flow-through systems with a flushing rate of 1–2 volumes per hour to maintain a constant anthracene concentration and minimise the accumulation of ^{14}C-labelled biotransformation products and other metabolites. Anthracene concentration was monitored throughout the study. Three or four replicate samples of 5 midges were taken after approximately 0·5, 1, 2, 4, 8, 12, 20 and 30 h. Because sampling intervals varied slightly among experiments, actual length of exposure was for computations.

In depuration experiments midges exposed to anthracene for 9 h were transferred to uncontaminated aerated water containing paper towel substrate. Paper towel was the substrate used for rearing the midges. This substrate could not be used for the uptake phase because of sorption and microbial degradation of anthracene on the paper towel. Three replicate samples of 5 midges and a sample of water were taken after 1, 2, 4, 8, 16, 24 and 30 h of depuration. Water was changed if activity was twice background.

Biotransformation rates of anthracene were measured at three temperatures (16, 25 and 30 °C) at 22 μg litre^{-1}. Four replicate samples of 30 midges were taken after 0·5, 1, 2 and 4 h. At 30 °C, samples were also taken after 9 and 18 h.

Extraction and analysis

Water samples were placed directly into the scintillation cocktail (3a70B Research Products International) for ^{14}C-activity determination. Midges

were desiccated overnight at room temperature in glass desiccation chambers and weighed on a model 4700 Cahn Electrobalance. Total ^{14}C in midges was collected into a 4 ml Carbosorb and 15 ml RPI 3a70B scintillation cocktail following combustion by a model 306 Packard sample oxidiser. Internal and external standards indicated ^{14}C recovery greater than 99% and no carry-over of ^{14}C.

Biotransformation products of anthracene were measured in undesiccated midges which had been blotted dry. Samples were stored in a 4:1 (v/v) solution of ethylacetate:acetone at -40°C until they were extracted. Samples were homogenised in a Ten Broeck homogeniser and extracted twice with 5 ml of 4:1 (v/v) ethylacetate:acetone and once with 5 ml of cyclohexane. The extracts were combined and filtered through Whatman No. 40 paper. The extract was evaporated to 0·5 ml under a stream of nitrogen. Activity was determined from a 50 μl aliquot. The filter paper was burned, counted, and reported as unextractable metabolite. Adsorbtion of dissolved ^{14}C by the filter paper was minimal. Mean ($\pm$SE) recovery of spiked samples was $88 \pm 5\%$ ($n = 5$).

Aliquots of 200 μl were spotted and chromatographed on precoated silica gel thin layer chromatography plates (E. Merck) in hexane:benzene (9:1 v/v) and pentane:ether (9:1 v/v). Developed plates were sectioned into 4 to 5 parts corresponding to the origin, anthracene, and 2 or 3 other spots visualised by UV light. Spots were scraped into RPI 3a70B for ^{14}C-activity determination. The origin, non-anthracene spots and filter paper are reported as total metabolite. Biotransformation rates were determined by dividing total metabolite by duration of exposure.

^{14}C activity of all samples was determined with a Beckman model LS 8100 liquid scintillation counter. The samples were corrected for background, quench and counting efficiency. The quench and counting efficiency were corrected using a quench curve and the samples channels ratio method. Counting efficiency for water and combusted insects was 85% and 87%, respectively.

Data analysis

Rate constants, ^{14}C-bioconcentration factors (^{14}C-BCF) and 95% confidence limits were estimated by Marquardt iterative least squares procedures (procedure NLIN, Statistical Analysis System, Barr *et al.*, 1979), with a one compartment donor dependent model (Hamelink, 1977; Giesy *et al.*, 1980).

Since K_u and K_d were estimated simultaneously by numerical methods, they were highly correlated. Bias introduced into one estimate by violating assumptions can affect the other. Therefore, we estimated K_u and K_d by several other techniques which made different assumptions.

Independent estimates of K_u were calculated from slopes of tangents to the uptake curve at 1 h. This initial rates technique assumes a first order relationship with respect to C_w and no initial depuration (K_u = slope · C_w). The initial rate estimate is influenced less by biotransformation and accumulation of bound materials, and more by handling, than estimates made using other techniques. A smaller sample size limits the precision of this estimate. The overall depuration rate constant (K_d) was also estimated from data collected during the clearance period, when midges were not exposed to anthracene. The overall depuration rate constant was estimated from the slope of the first log-linear component of the depuration curve. The first component contains contributions from the depuration rate constants from each pool of contaminant within the organism. Subsequent log-linear components result after depletion of more rapidly eliminated pools. Depuration rate constants were estimated by the method of Wagner (1975).

RESULTS AND DISCUSSION

Uptake

Uptake of ^{14}C-anthracene was rapid and approached steady state in 25 to 45 h (Fig. 1). The uptake rate constant for ^{14}C increased over the concentration range 1·7 to 30·5 μg litre^{-1}.

K_u based on ^{14}C generally decreased as exposure time increased (Table 1). Since K_u and K_d were estimated simultaneously from ^{14}C data, they may be biased by accumulation of stored biotransformation products (see sections on depuration and biotransformation).

The decrease of K_u may also be due to a decline in swimming and activity. The speed of the normal swimming motion of midges decreased as duration of exposure increased. Midges in uncontaminated water also showed the same activity patterns. Slower swimming activity increases the boundary layer of less concentrated anthracene and thus reduces exposure concentration. Respiration and metabolic rates, as manifested in swimming rates (Walshe, 1949) could also influence uptake rate K_u

TABLE 1

Uptake (K_u) and Depuration (K_d) Rate Constants in *C. riparius* at Four Concentrations and Three Temperatures (Estimate ± 95% CI)

Concentration $\mu g\ litre^{-1}$	*Temperature* (°C)	$K_u\ (h^{-1})$			$K_d\ (h^{-1})$			
		1 h[a,b]	*10 h*[c]	*30 h*[c]	*10 h*[c]	*30 h*[c]	*3 h*[d]	*30 h*[e]
1·7	25	82	77 ± 21	68 ± 13	0·070 ± 0·073	0·035 ± 0·019	0·077 ± 0·023	0·311 ± 0·224
8·7	25	79	54 ± 13	74 ± 10		0·041 ± 0·014	0·215 ± 0·078	0·236 ± 0·098
22·3	25	193	161 ± 12	155 ± 16	0·107 ± 0·021	0·085 ± 0·014	0·158 ± 0·176	0·247 ± 0·101
30·5	25	255	222 ± 32	143 ± 20	0·276 ± 0·070	0·078 ± 0·017	0·093 ± 0·014	0·177 ± 0·120
22·2	16	128	116 ± 10	102 ± 11	0·091 ± 0·023	0·053 ± 0·011	0·144 ± 0·027	0·250 ± 0·457
22·3	25	193	161 ± 12	155 ± 16	0·107 ± 0·021	0·085 ± 0·014	0·158 ± 0·018	0·247 ± 0·102
22·7	30	153	152 ± 14	152 ± 16	0·089 ± 0·026	0·089 ± 0·015	0·266 ± 0·139	0·363 ± 0·231

[a] Duration of test. All values estimated from concentration of ^{14}C-anthracene in midges (ng g^{-1} wet wt).

[b] Estimated from tangent to slope of 1 h.

[c] Estimated from ^{14}C-anthracene uptake experiments using donor dependent model.

[d] Measured as slope during initial 3 h in depuration in uncontaminated water and paper towel substrate after 9 h exposure (depuration experiment).

[e] Depuration rate from most rapidly eliminated pool (Wagner, 1975, p. 58) (depuration experiment).

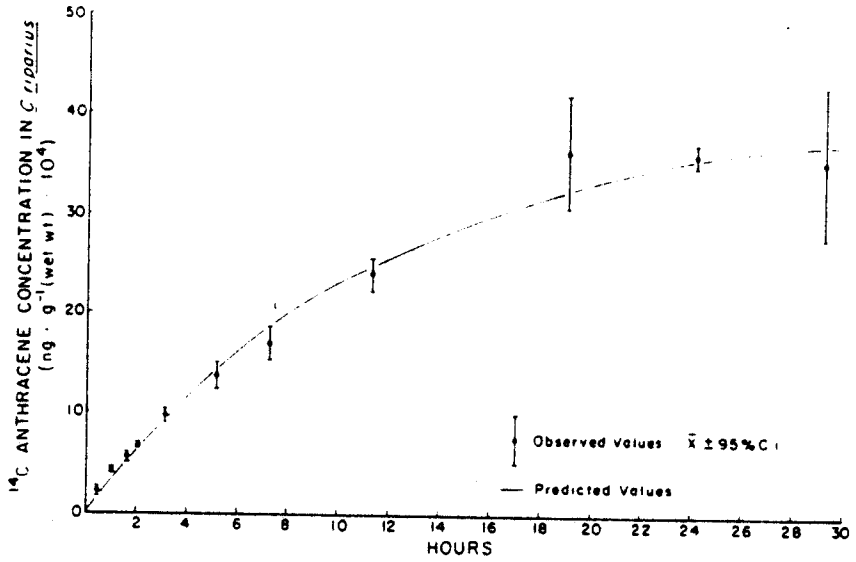

Fig. 1. Accumulation of ^{14}C anthracene by *C. riparius* at 25 °C and 22 μg litre^{-1}. Each point represents the mean ± 2 SE of 3 replicates.

values measured at 25 and 30 °C were not significantly different, but were higher than those determined at 16 °C (Table 1).

Depuration

Depuration rate constants estimated from 10 h uptake experiments were greater at higher anthracene concentrations (Table 1). As exposure time increased, a larger fraction of the ^{14}C was in the bound or unextractable pools and a smaller proportion of the ^{14}C material was available for depuration. K_d was estimated from ^{14}C material, not from the unbound fraction that was available for depuration. Therefore K_d increasingly underestimated depuration of unbound material, and decreased with time. Polar biotransformation products are eliminated more slowly than are the original compounds by other aquatic animals (Landrum & Crosby, 1981).

In all but one time period (30 h, 16 °C) there was no significant difference in depuration with respect to temperature. The difference was small and not considered biologically significant. It was not reflected by the general trend in any of the other methods for estimation of K_d.

Temperature and concentration did not affect K_d estimates from depuration experiments. K_d from uptake experiments were greater than K_d from depuration experiments. One of the differences between uptake and depuration experiments was that depuration experiments used paper towel and uptake experiments used none. Leversee *et al.* (1982) observed greater rates of depuration of benzo(a)pyrene (BaP) from *C. riparius* in uncontaminated water in the presence of paper towel than in the absence of paper towel.

We observed multiphasic depuration of ^{14}C-anthracene (Fig. 2). The phases may represent depuration of anthracene from several physiological compartments, or depuration of anthracene and its biotransformation products which are more polar. Resolution of these questions would be difficult in the midge and our studies were not designed to resolve them.

After the two depuration phases, a residual of approximately 20 % of the initial ^{14}C still remained bound in the animal at 30 °C (Fig. 2). This residual ^{14}C was depurated very slowly or not at all. During this final phase the slope of the log-transformed ^{14}C concentration as a function of time was not significantly different from zero. Residual material in midges may be carried into the terrestrial environment by emerging adults

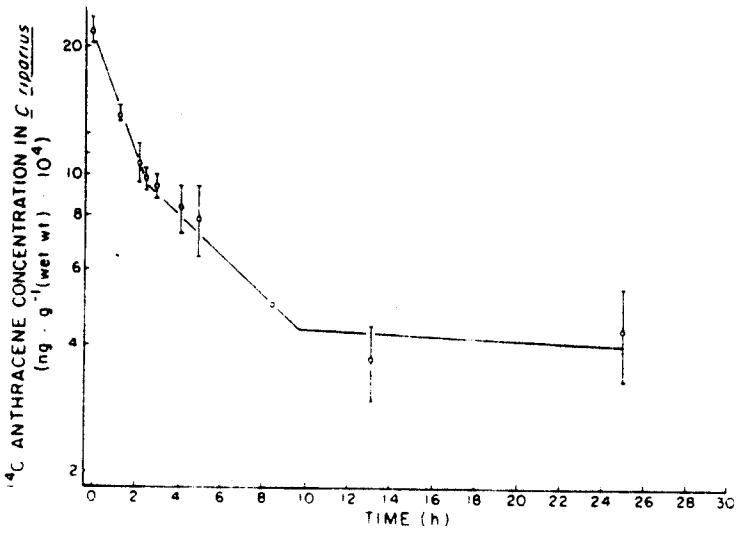

Fig. 2. Depuration of ^{14}C-anthracene by *C. riparius* in uncontaminated water and paper towel substrate at 30 °C. $\bar{X} \pm 2$ SE, $n = 3$.

(Menzie, 1980). In work with *Chironomus tentans*, Derr & Zabik (1972) found that most DDE in larvae and pupae was retained when the midge pupated and emerged as a terrestrial adult.

Biotransformation

The biotransformation rate was maximum at 25 °C (Figs 3 and 4). Because the biotransformation rate was high, percent anthracene was minimum at 25 °C. The mass of anthracene in the midges was maximum at 30 °C because accumulation of ^{14}C was high, but biotransformation was lower at 30 °C than at 25 °C (Fig. 5).

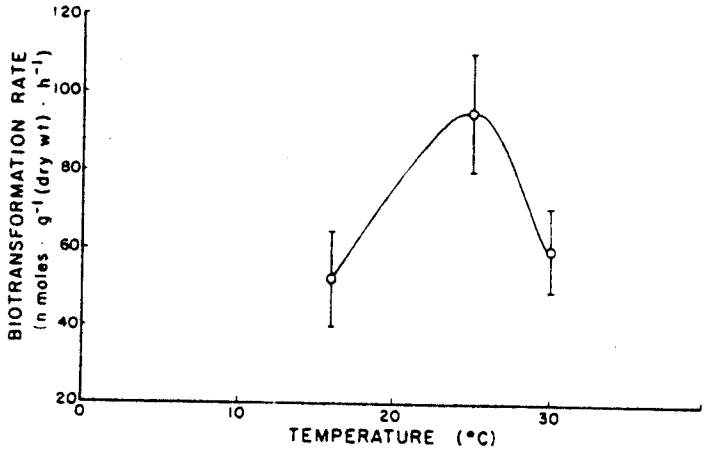

Fig. 3. Biotransformation rate after 1 h exposure as a function of temperature. $\bar{X} \pm 2SE$, $n = 4$.

The overall biotransformation rate was slower at 16 °C than at higher temperatures (Fig. 5). The proportion of biotransformed anthracene that was unextractable was lowest at 16 °C.

At 30 °C, the percent of ^{14}C as anthracene decreased with length of exposure from 48 % at 0·5 h to 9 % at 18 h (Fig. 4). The trend was less pronounced at the other temperatures. Between 4 and 18 h, anthracene concentration decreased by two-thirds while the ^{14}C activity rose by a factor of 2·7. However, at all temperatures percent unextractable ^{14}C increased with time.

Decreased biotransformation rates during the first 4 h (Fig. 5) may

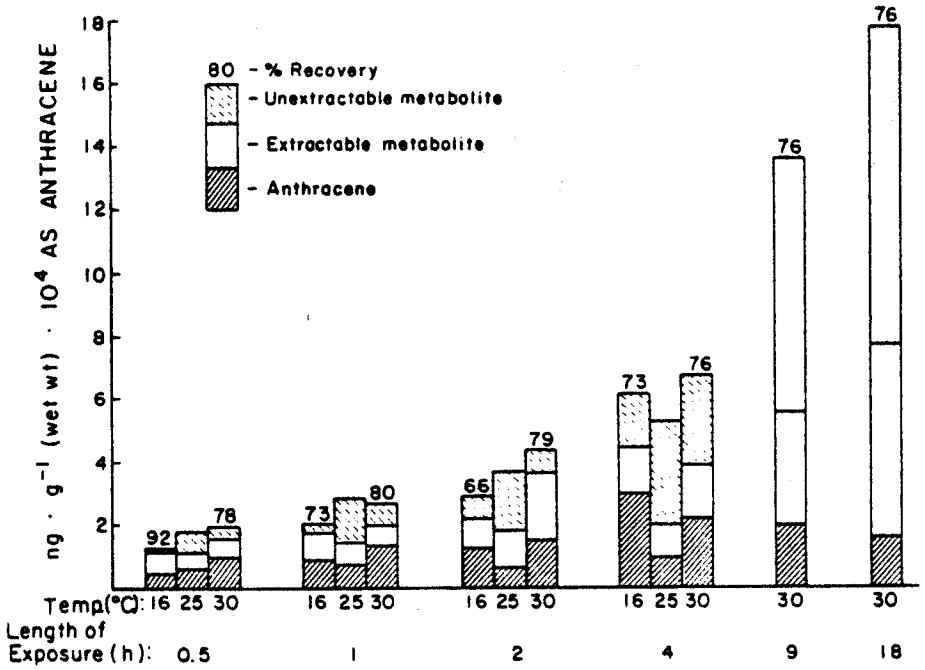

Fig. 4. Biotransformation rate as a function of time of exposure and temperature $\bar{X} \pm 2SE$. $n = 4$.

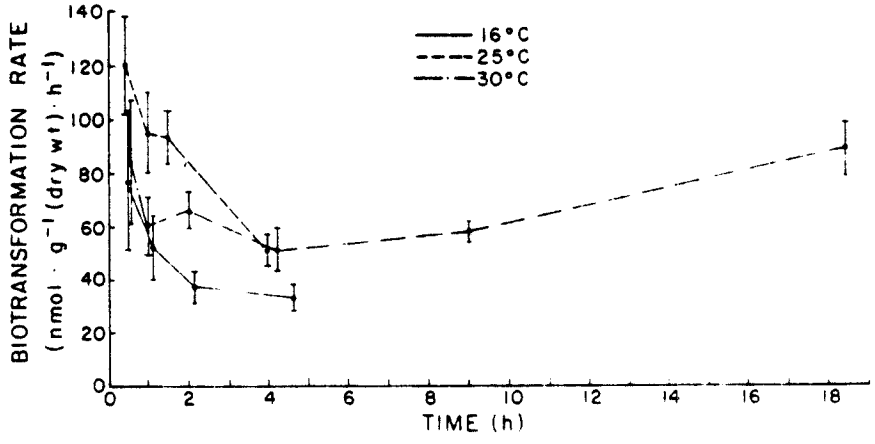

Fig. 5. Biotransformation of ^{14}C anthracene at 16, 25 and 30 C. $\bar{X} \pm 2SE$. $n = 4$

have been due to a decline in respiration, as discussed earlier, or to a build-up of biotransformation products producing end-product inhibition (Chen *et al.*, 1979). Leversee *et al.* (1982) found a similar decrease in biotransformation rate in midges exposed to B(a)P. However, at 30 °C biotransformation rate tended to increase after 4 h (Fig. 5).

Bioconcentration factor

Bioconcentration factor (^{14}C-BCF), calculated using $K_u \cdot K_d^{-1}$ based on ^{14}C at 30 h, was not affected by temperature or concentration of anthracene in the water (Table 2). Other studies with aquatic organisms

TABLE 2

Bioconcentration Factors (BCF) for ^{14}C-Anthracene in the Midge *C. riparius*. BCF for Total ^{14}C was Calculated from $K_u \cdot K_d^{-1}$ after 10 and 30 h of Uptake. BCF for Anthracene was Calculated from Concentrations of Anthracene at 4 h ($\bar{X} \pm 95\%$ CI)

Concentration ($ng\,ml^{-1}$)	*Temperature* (°C)	*Bioconcentration factors* ^{14}C		*Anthracene*
		10 h	*30 h*	*4 h*
1·7	25	915 ± 532	1 964 ± 722	
8·7	25		1 817 ± 388	
22·3	25	1 503 ± 206	1 820 ± 136	
30·5	25	804 ± 104	1 843 ± 180	
22·2	16	1 277 ± 245	1 915 ± 228	132 ± 39
22·3	25	1 503 ± 206	1 820 ± 136	47 ± 15
22·7	30	1 697 ± 384	1 702 ± 119	95 ± 5

(Wilkes & Weiss, 1971; Blanchard *et al.*, 1977; Canton *et al.*, 1978) have likewise shown that BCF was constant at different concentrations. Similarly, bioaccumulation was found to be unaffected by temperature in *Mytilus edulis* (Fossato & Canzonier, 1976). Other authors have observed a negative correlation between temperature and uptake or retention of trace organic compounds in coho salmon, copepods (Collier *et al.*, 1978) and clams (Fucik & Neff, 1977; Harris *et al.*, 1977).

The BCF calculated for anthracene in biotransformation studies was more than an order of magnitude lower than ^{14}C-BCF, due to rapid biotransformation of anthracene. Interestingly, at 30 °C, the BCF for anthracene at 18 h was 68 ± 17, which is two-thirds of the concentration

factor at 4 h. Anthracene in the midges did not remain at its maximum concentration. Hence the assumption of an approach to steady state at the maximum anthracene concentration was not supported by the data. Although anthracene concentration fell after 4 h, ^{14}C continued to bioaccumulate. Thus the donor-dependent model accurately predicted the changes in ^{14}C concentration over time, but did not predict decreases in anthracene concentration in the organism. This type of model is of limited usefulness for prediction of parent compound concentration when site saturation and biotransformation are important. Anthracene concentration factor was lowest at 25 °C and reflected the biotransformation rate.

BCF was more strongly affected by differences in biotransformation rate due to temperature than by differences in uptake rate due to temperature. For example, BCF was highest at 16 °C because biotransformation was lowest, even though uptake rate and anthracene concentration in the animal were also lowest at 16 °C. As we found with anthracene in chironomids, ^{14}C-BCF overestimates bioconcentration. Consequently, caution must be exercised in comparing BCF from total ^{14}C data with those from direct compound analysis of metabolically-transformable compounds.

Benzo(a)pyrene and anthracene

Since the octanol:water partition coefficient has been positively correlated with BCF (Neely *et al.*, 1974), benzo(a)pyrene (BaP) would be expected to have a larger BCF than anthracene. Accordingly, the 4 h accumulation factor based on parent compound was approximately 4 times greater for BaP (200) than for anthracene (47). However, the ^{14}C-BCF of anthracene (915 at 1·7 ng ml^{-1}) was similar to ^{14}C-BCF for BaP in *C. riparius* (970 at 1·0 ng ml^{-1}) (Leversee *et al.*, 1982). Though both K_u and K_d were slower for anthracene, the ^{14}C-BCF values were similar for anthracene and BaP because their relative proportions remained constant. The difference between BCF based on ^{14}C and BCF based on parent compound for anthracene compared respectively with those for BaP indicates a slower elimination of anthracene biotransformation products. Relative rates of biotransformation on a molar basis were similar for BaP (2·7 nmol g^{-1} h^{-1} at 4 nmol litre^{-1}) and anthracene (51 nmol g^{-1} h^{-1} at 125 nmol litre^{-1}) considering the difference in concentration.

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