

RELATIONSHIP OF TOXICOKINETIC PARAMETERS TO RESPIRATION RATES IN *MYSIS RELICTA*

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ABSTRACT. The uptake of organic xenobiotics from water was compared to the respiration rate of the Great Lakes invertebrate, *Mysis relicta*. Xenobiotic clearance was compared with oxygen clearance. Uptake clearance is defined as milliliters of water stripped of contaminant or oxygen per mass of organism per hour. *M. relicta* were exposed to benzo(a)pyrene (BaP) and 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP). The respiration rate for *M. Relicta* declined logarithmically when measured for periods ranging from 3 to 48 h. The respiration rate ranged from 1.4 (1.24–1.48) $\mu\text{g O}_2 \text{ mg}^{-1} \text{ wet weight h}^{-1}$ for a 3 h period to $0.25 \pm 0.023 \mu\text{g O}_2 \cdot \text{mg}^{-1} \text{ wet weight} \cdot \text{h}^{-1}$ for a 48 h period. Neither the presence of the methanol carrier nor the methanol carrier plus xenobiotics produced significant changes in respiration compared to those of the control organisms in lake water only. The clearances of oxygen and the two xenobiotics were each inversely correlated to organism weight. The slopes of the clearance regressions with organism weight were not significantly different among the different measures of xenobiotic or oxygen clearance. There was a near constant ratio of xenobiotic clearance to oxygen clearance of 1.25 ± 0.01 (mean \pm SE, $n=29$) for BaP and 1.37 ± 0.12 ($r = 29$) for HCBP. These findings suggest that the accumulation of organic xenobiotics may be linked to the accumulation of oxygen for *M. relicta*.

INDEX WORDS: Respiration, toxicokinetics, *Mysis relicta*, benzo(a)pyrene, hexachlorobiphenyl.

INTRODUCTION

The respiratory surfaces of aquatic animals, e.g., gill surfaces for fish, are considered the major point of xenobiotic uptake particularly for nonpolar organic xenobiotics from water (Spacie and Hamelink 1982). In fish, the ratio of oxygen clearance to that for xenobiotics varies from 30 to 161% (Norstrom *et al.* 1976). Clearance is defined as the milliliters of water stripped of substrate, in this case oxygen, per mass of organism per hour. More recent work with fish has found that the efficiency of both oxygen and xenobiotic transfer is essentially the same for nonpolar organic xenobiotics that have log octanol:water partition coefficients ($\log K_{ow}$) in the range of 3 to 6.5 (McKim *et*

al. 1985). For invertebrates, the efficiency of uptake for nonpolar xenobiotics compared to oxygen uptake was found to range from 5% for *Mysis relicta* (Frez and Landrum 1986) to 300% for *Stylodrilus heringianus* (Frank *et al.* 1986) based on published values for oxygen consumption.

One study has simultaneously measured oxygen clearance and xenobiotic uptake in invertebrates and found that xenobiotic uptake clearance was 3.8 to 4.2 times greater than oxygen clearance for the Great Lakes amphipod, *Diporeia* spp. (Landrum and Stubblefield 1991). Reasons for the large variability between xenobiotic clearances and oxygen clearance among the various invertebrates remain unclear. However, the variation in the relative clearance of oxygen compared to xenobiotics

may result from the low oxygen uptake efficiencies of some organisms such as amphipods (Sutcliffe 1984) or to the potential additional routes for xenobiotic accumulation in some species, e.g., accumulation of xenobiotics across the chiton exoskeleton (Landrum and Stubblefield 1991). Why *M. relicta* xenobiotic clearance is so low compared to oxygen clearance remains to be determined. However, if bio-energetic approaches for xenobiotic uptake are to be applied to xenobiotic uptake models for aquatic invertebrates, adequate baseline laboratory data relating respiration rates and xenobiotic clearances in aquatic invertebrates must be obtained.

The objective of this study was to examine the relationship between oxygen consumption and xenobiotic uptake in *M. relicta*. *M. relicta* is an integral component of the Great Lakes food web. This animal is a major predator (Bowers and Vanderploeg 1982, Bowers and Grossnickle 1978) and prey (Wells and Beeton 1963, Dryer *et al.* 1965) species in the Great Lakes. To examine the relationship between respiration and xenobiotic accumulation, a representative polychlorinated biphenyl (PCB) congener, 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP), and a polycyclic aromatic hydrocarbon (PAH) congener, benzo(a)pyrene (BaP), both with relatively large and similar K_{ow} values, were selected. Both of these compounds are important Great Lakes xenobiotics (Nriagu and Simmons 1984) that can potentially bioconcentrate in aquatic invertebrates and are useful surrogates to assess the movement of important classes of xenobiotics in aquatic environments. As such, an understanding of the xenobiotic dynamics in this species may aid in understanding the dynamics of such chemicals within the Great Lakes ecosystem.

METHODS AND MATERIALS

Mysis Collection

Fourth and fifth instar *M. relicta*, estimated by the length-weight relationships reported by Reynolds and DeGraeve (1972), were collected at night from Lake Michigan, 5 miles west of Grand Haven, Michigan, by towing a 1.0 x 3.0 m nitex net (565 μm mesh) vertically through the water column. The animals were transferred to 50-L insulated containers filled with lake water held at 4°C and transported to the laboratory within 18 hours. At the laboratory, *M. relicta* were transferred to an

aquarium containing 175 L lake water. *M. relicta* were fed daily rations of trout chow.

Compounds and Radiopurity

³H-Benzo(a)pyrene (BaP, specific activity = 23.8 Ci mMol⁻¹) was obtained from Amersham while ¹⁴C-2,2',4,4',5,5'-hexachlorobiphenyl (HCBP, specific activity = 13.1 mCi mMol⁻¹) was obtained from Pathfinder Laboratories (currently Sigma Radiochemicals). The compounds were tested for radiopurity with a combination of thin layer chromatography and liquid scintillation spectrometry. The compounds were chromatographed on silica gel plates (250 μm , E Merck) in hexane:benzene (8:2 V:V). The silica gel was scraped from the plates at the retention position corresponding to the parent compound and several other segments including the origin. Each segment was placed in a separate scintillation vial and 12 mL scintillation cocktail (3a70B, Research Products International) added. The radioactivity of the samples was determined on a Packard 460C scintillation spectrometer. The radiopurity was determined as the percent of activity for the parent compound relative to the total activity for the sample. All compounds used for study were at least 98% radiopure. When compounds were found to be less than 98% pure, they were purified by thin layer chromatography using the same solvent system as above.

Respiration Measurements

All experiments were conducted using lake water filtered through glass fiber filters (Gelman A/E, nominal pore size 1 μm) to minimize the potential oxygen demand of suspended materials. Additionally, all experiments were started at the same time of the day to minimize the effects of diurnal variability on the experimental results. The water was added to fill the BOD bottles and cooled to 4°C. Single animals were added to the bottles. The bottles were then stoppered and placed in the dark for the designated time period. Oxygen measurements were made using the Winkler titration method (Grasshoff 1983) with the following modifications: the sodium thiosulfate titrant concentration was reduced from 0.01 N to 0.005 N to improve the sensitivity of the titration end point and was standardized daily against freshly made KIO₃. Reagent background and blanks (using nitrogen-purged lake water) were determined daily. Triplicate oxygen concentrations were determined at the beginning of the experiments. After the designated time

period, each mysid was removed while taking care not to aerate the water, blotted dry, and weighed on a Cahn electro-balance (Model 4700). (Note: the dry/wet weight ratio is 0.15 ± 0.04 , mean \pm SD, $n = 9$.) The water was fixed with Winkler reagents and placed in the dark at 4°C until the hydroxide flock had settled (approximately 1 h). Then, the samples were acidified with H_2SO_4 , and 50-mL aliquots were titrated in triplicate with the standardized sodium thiosulfate. The titration precision had a coefficient of variation between 0.1 and 0.5%. The oxygen concentrations in bottles without mysids were also determined at the end of the time period as controls for oxygen losses not accounted for by the mysids. The final control oxygen concentrations did not differ from initial oxygen concentrations. The amount of oxygen consumed by each animal was determined by the difference between the oxygen concentrations in the control bottles and those containing mysids. Average respiration rates (RO_2) were calculated using the equation:

$$\text{RO}_2 = (([\text{O}_2]_c - [\text{O}_2]_m) \cdot V) / (M \cdot t) \quad (1)$$

where

- $[\text{O}_2]_c$ = the oxygen concentration ($\mu\text{g mL}^{-1}$) of the control,
- $[\text{O}_2]_m$ = oxygen concentration ($\mu\text{g mL}^{-1}$) of the water in the bottles containing mysids,
- M = mysid wet weight (mg),
- t = time (h), and
- V = volume of water in the BOD bottle (mL).

The average oxygen clearance (CLO_2) was then calculated using the equation:

$$\text{CLO}_2 = \text{RO}_2 / [\text{O}_2]_a \quad (2)$$

where

- CLO_2 = the average clearance ($\text{mL g}^{-1} \text{h}^{-1}$) of oxygen for the designated time and is the conditional removal rate of O_2 for the mysid;
- $[\text{O}_2]_a$ = the average oxygen concentration ($\mu\text{g mL}^{-1}$) between the initial and final concentrations;
- RO_2 = the respiration rate as calculated above expressed in units of $\mu\text{g O}_2 \text{g}^{-1} \text{h}^{-1}$.

Experiments were conducted to test the effects of three different bottle volumes on respiration rates. In these experiments, filtered lake water at

4°C was added to 65-mL BOD bottles, 300-mL BOD bottles, and 700-mL screw cap serum bottles, which were all previously cooled to 4°C . Mysids were then placed in the bottles, and the bottles were capped and placed in the dark at 4°C for 12 h. Respiration rates were then measured as described above.

The kinetics of respiration rates were measured for periods of 3, 6, 12, 24, and 48 h. In these kinetic experiments, 300-mL bottles were filled with filtered lake water at 4°C . Mysids were then added and the bottles were then stoppered and placed in the dark at 4°C . Respiration rates were then determined as above.

To examine the potential effects of xenobiotics on respiration rate, *M. relicta* were exposed to five different environments: BaP in methanol, HCBP in methanol, BaP and HCBP in methanol, methanol alone, and filtered lake water. Quintuplicate analyses for each treatment were performed. This approach also permitted the investigation of the relationships between oxygen and xenobiotic clearance.

Xenobiotic Clearance Estimates

Average xenobiotic clearance rates were determined simultaneously with the respiration experiments using a dual labeling method. HCBP (concentration range 1.1 to $1.6 \mu\text{g L}^{-1}$) and/or BaP (concentration range 2.6 to 4.1 ng L^{-1}) in a methanol carrier (concentration range 0.18 to 0.16 mL L^{-1}) was added to the filtered lake water cooled to 4°C . The dosed lake water was then added to 300-mL BOD bottles. Triplicate initial water xenobiotic concentrations were measured for 2-mL water samples from each of three control bottles. All xenobiotic concentrations were determined from the measured radioactivity of the samples and calculated from the known specific activity. Mysids were placed in three test bottles which were stoppered and stored at 4°C in the dark for 24 h. After 24 h, the mysids were removed from the bottles, blotted dry, weighed, and placed in 12 mL of liquid scintillation cocktail. To measure residual xenobiotic concentration, 2-mL water samples were taken at the end of the exposures and placed in 12 mL cocktail. The concentrations of the xenobiotic that were freely dissolved and that bound with dissolved organic matter were determined using reverse phase separation (Landrum *et al.* 1984). The BaP and HCBP concentrations in animals and water were determined from the respec-

tive amount of ^3H and ^{14}C activity in the various samples. Radioactivity was measured on a Packard 460C liquid scintillation spectrometer, using an external standard ratio method to correct for quenching after subtracting background. The average xenobiotic clearance coefficient (k_w) was calculated using a mass balance model with the following equation (Landrum 1983):

$$k_w = (-\ln[1 - Q_a/Q_t])/t(V/M) \quad (3)$$

where

k_w = the clearance coefficient ($\text{mL g}^{-1} \text{h}^{-1}$),
 Q_a = quantity (ng) of toxicant in the animal,
 Q_t = total quantity (ng) of toxicant in the system,
 V = volume (mL) of water in the bottle,
 M = wet weight (mg) of the mysids, and
 t = time (h).

The above equation assumes that elimination is unimportant over the course of the accumulation. In the absence of feeding, the elimination of BaP and HCBP is very slow and would not be significant over the time course of these studies (Frez and Landrum 1986). The advantage of this model which takes mass balance into consideration is that the xenobiotic clearance can be estimated from a knowledge of the quantity of xenobiotic in the animal and in the system and the duration of exposure. Since the focus of the calculation is on the rate of accumulation, whether the compound exists as parent compound or metabolite is unimportant. The amount of compound in the organism was calculated from the specific activity. *M. relicta* metabolize BaP significantly but not HCBP (Gardner *et al.* 1990).

Statistics

Regressions were performed using the statistical programs in SYSTATTM (Wilkinson 1988). Comparisons between means and slopes were accomplished using Student's *t* test and differences were considered significant when $p < 0.05$.

RESULTS

Respiration

After 24 h, the maximum respiration rate ($0.79 \pm 0.23 \mu\text{g O}_2 \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$, $n = 3$) was found for animals housed in 300-mL bottles compared to those housed in 65- ($0.23 \pm 0.04 \mu\text{g O}_2 \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$, $n = 6$) and 700-mL bottles ($0.42 \pm 0.01 \mu\text{g O}_2 \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$,

$n = 3$). Therefore, the 300-mL bottles were chosen for the remaining tests because it was possible to make replicate measures of oxygen concentration from each bottle and the volume was sufficiently small so that measurements would not be near the limit of detection.

The mysid respiration rates were at least four times larger at 3 h than at 48 h (Fig. 1). Between 3 and 48 h, the respiration rate exhibited an apparent log-linear decline and reached an apparent asymptote between 24 and 48 h. Because the 24 h respiration measure was near the asymptote, and for comparison with published data, all comparisons of xenobiotic and oxygen clearances are reported for 24 h duration.

The mean lake water respiration rates for *M. relicta* (Table 1) are similar to literature values reported for *M. relicta*: $0.2482 \mu\text{g O}_2 \text{ mg}^{-1} \text{ wet weight h}^{-1}$ at 4°C (Foulds and Rolf 1976) and $0.4771 \mu\text{g O}_2 \text{ mg}^{-1} \text{ wet weight h}^{-1}$ at 5°C (Lasenby and Langford 1972). Overall, the mean respiration rates for mysids exposed to the BaP and/or HCBP appeared to be slightly higher than for mysid respiration in lake water only (Table 1). However, the respiration rate depends on the size of the animals, thus mean values can be misleading and the contribution of size to the variance must be considered. While such size-related variance in respiration is

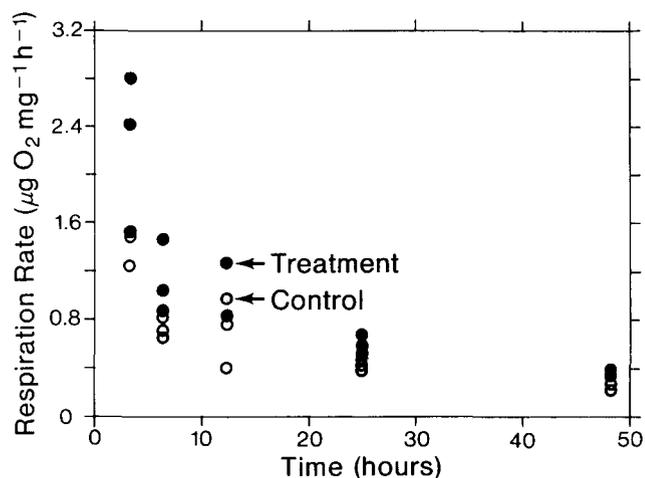


FIG. 1. The effect of experimental duration on the respiration rate of *Mysis relicta* exposed to lake water only (controls) and a mixture of benzo(a)pyrene and hexachlorobiphenyl using a methanol carrier. The initial oxygen concentration was $7.36 \pm 0.07 \mu\text{g O}_2 \text{ mL}^{-1}$ initially and declined to $5.58 \pm 0.31 \mu\text{g O}_2 \text{ mL}^{-1}$ at the end of 48 h.

TABLE 1. Respiration rates of *M. relicta* exposed to various combinations of xenobiotics.

Treatment	Respiration Rate ¹ $\mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$	N (no. of replicates)
Lake Water	0.186 ± 0.071	15
BaP, HCBP, MEOH	0.209 ± 0.070	15
BaP, MEOH	0.239 ± 0.090	15
HCBP, MEOH	0.265 ± 0.089	14
MEOH	0.220 ± 0.053	11

¹Mean \pm SD

well recognized (Prosser 1973), the usual form of the relationship is a power function. In this case, the data are fit equally well by a log-linear regression or by a simple linear regression. The data fit a linear function because of the relatively small size range employed in the regression: RO_2 ($\mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$) = $-0.0021 (\pm 0.0004) \cdot \text{mass (mg)} + 0.344 (\pm 0.022)$ ($n = 69$, $r^2 = 0.33$, $p < 0.001$) and are represented by the linear relation of oxygen clearance versus weight (Fig. 2). The relationship between oxygen clearance and respiration is given by the following: RO_2 ($\mu\text{g O}_2 \text{ mg}^{-1} \text{ h}^{-1}$) = $0.0061 (\pm 0.0003) \cdot \text{Clearance (mL g}^{-1} \text{ h}^{-1}) + 0.004 (\pm 0.01)$ ($n = 69$, $r^2 = 0.88$, $p < 0.001$). When the various experimental groups, controls, carrier controls, and xenobiotics plus carrier were analyzed separately there were no statistical differences in their slopes or intercepts. This indicates that neither the presence of BaP and HCBP with the methanol carrier nor the methanol carrier alone affects the respiration of *M. relicta*.

Xenobiotic Clearance

If xenobiotic and oxygen clearances are similarly related to the metabolic rate of the animal, they may also be related to each other. If so, xenobiotic clearance should also be related to morphometric measurements that may reflect metabolic rates, such as mass and surface area. In this study, the clearances of oxygen, HCBP, and BaP are all related to organism size, and the regression lines of clearance versus organism mass all have slopes that are not statistically different (Fig. 2). The intercept for the clearance of oxygen is about half that of the two xenobiotics. The clearance rates for the two xenobiotics were strongly correlated (Fig. 3). However, where the clearance of oxygen and one of the xenobiotics was measured for the same organisms, the oxygen clearance was not a good predictor of

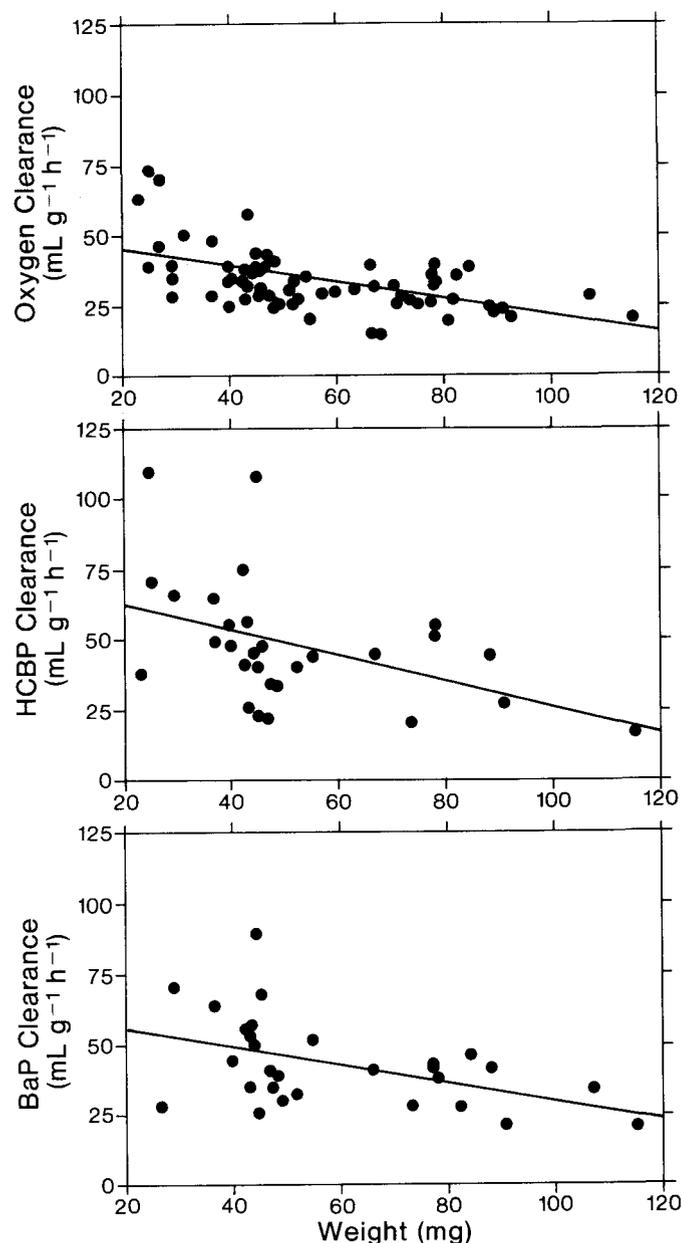


FIG. 2. The relationships for oxygen, hexachlorobiphenyl, and benzo(a)pyrene clearances ($\text{mL g}^{-1} \text{ h}^{-1}$) to *Mysis relicta* mass (mg). Oxygen clearance = $51.7 (\pm 3.6) - 0.28 (\pm 0.06) \cdot \text{mass (mg)}$ ($n = 69$, $r^2 = 0.24$, $p < 0.001$); BaP clearance = $61.9 (\pm 7.3) - 0.32 (\pm 0.15) \cdot \text{mass (mg)}$ ($n = 29$, $r^2 = 0.23$, $p < 0.01$); HCBP clearance = $71.5 (\pm 9.8) - 0.45 (\pm 0.18) \cdot \text{mass (mg)}$ ($n = 29$, $r^2 = 0.20$, $p < 0.02$).

the xenobiotic clearance. This seemed to occur largely because of a half dozen outliers in the data sets. These data were all confined to one experi-

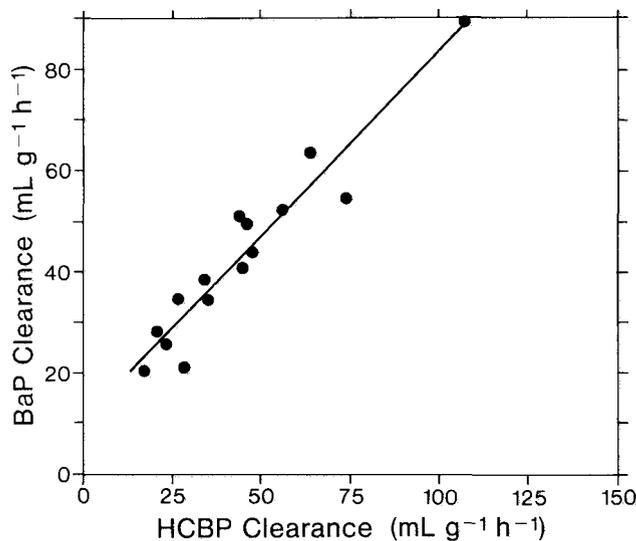


FIG. 3. The relationship between benzo(a)pyrene and hexachlorobiphenyl uptake clearances. BaP clearance = $11.0 (\pm 3.1) + 0.72 (\pm 0.06) \cdot \text{HCBP clearance}$ ($n = 15$, $r^2 = 0.91$, $p < 0.001$).

ment and there were no apparent experimental difficulties. However, if the data from that one experiment are eliminated there are significant relationships between oxygen clearance and xenobiotic clearance (k_w (BaP) = $0.79 (\pm 0.19) \cdot \text{CLO}_2 + 8.8 (\pm 6.7)$, $n = 18$, $p < 0.001$, $r^2 = 0.52$; k_w (HCBP) = $0.69 (\pm 0.21) \cdot \text{CLO}_2 + 11.3 (\pm 8.2)$, $n = 18$, $p < 0.004$, $r^2 = 0.41$). The slopes of the regression lines are not significantly different and the intercepts are not significantly different from zero. Thus, the xenobiotic and oxygen clearances may not be entirely uncoupled because: 1) there is a similarity of the slopes of the mass-clearance relationships for the xenobiotics and oxygen; 2) if the one experiment is eliminated there is a significant relationship between oxygen clearance and the respective xenobiotic clearance; and 3) the mean ratio between the xenobiotic clearances and oxygen clearance, where both were measured in the same organisms, are 1.25 ± 0.1 (mean \pm SE, $n = 29$) for BaP and 1.37 ± 0.12 (mean \pm SE, $n = 29$) for HCBP. The small data sets, the large variability in the data, and some unexplained difficulty with one of the experiments may account for the absence of a significant correlation between the oxygen clearance and the xenobiotic clearances when all the data are considered.

DISCUSSION

Kinetic Effects on Respiration

Interestingly, the respiration rates for *M. relicta* do not stabilize until at least 24 hours after the start of the experiment. Similar kinetic results have been obtained for the amphipod *Gammarus pulex* (Sutcliffe 1984) and the Great Lakes amphipod, *Diporeia* (Landrum, P. F., unpublished data). Elevated respiration rates during the early portion of the kinetic analyses are likely due to increased activity of the animal. Indeed, respiration rates for *Gammarus oceanus* are proportional to the activity of the animal (Halcrow and Boyd 1967). Further, experiments with the amphipod, *Diporeia*, designed to examine the effect of handling on respiration, demonstrated that the high respiration rates observed initially could likely be attributed to handling stress, but the effect was short-lived and not observable after 24 h (Landrum, P. F., unpublished data). Because handling is unavoidable in any study using animals, these kinetic observations have important consequences for those making respiration rate measurements. If a time point is selected which is too close to the experimental time zero, the variability in the measurement may be extremely large thus confounding the precision and accuracy of the test. Moreover, interlaboratory comparisons of data may be difficult if various experimental durations are involved.

Alternatively, the reduced respiration rates at the latter portion of the experiment might be the result of respiratory conformation to reduced oxygen tensions. However, this is unlikely for mysids because respiration rates in *M. relicta* are not affected until oxygen concentrations fall below 10% saturation (Maucheline 1980). For all the tests in this study, oxygen concentrations were approximately 6–8 mg L⁻¹, which at 4°C ranges from 50–67% saturation. Thus, selection of 24 h experimental duration should eliminate most of the potential effects due to handling because the measured rate is near the asymptote.

Potential Toxic Effects of BaP, HCBP, and Methanol

The respiration rate can be influenced by many environmental and physiological factors and by specific toxic xenobiotics. Slight reductions in respiration were observed for the Great Lakes invertebrate, *Diporeia*, when exposed to small amounts of methanol after accounting for the mass depen-

dence of the respiration rate (Landrum and Stubblefield 1991). No such response was found for *M. relicta*. Similarly, the polycyclic aromatic hydrocarbons have been observed to reduce respiration at relatively high concentrations in the $\mu\text{g mL}^{-1}$ range (Tatem 1977, Harmon and Sanborn 1982, Sanborn 1982, Crider *et al.* 1982, Russell and Fingerman 1984, Geiger and Buikema 1981). These observed reductions in respiration were found at much higher concentrations than those employed for BaP in this study. Thus, it was not surprising that no reduction in respiration was observed for mysids exposed at low pg mL^{-1} concentrations. This result is consistent with the absence of any effects for the Great Lakes amphipod, *Diporeia*, exposed to low concentrations of polycyclic aromatic hydrocarbon and polychlorinated biphenyl congeners (Landrum and Stubblefield 1991).

Effect of Mysid Size on Respiration

The respiration for *M. relicta* exhibited the expected reduction with increasing organism mass, although the relationship, as represented by oxygen clearance versus weight, was linear rather than the usual power relationship (Fig. 2). The linearity of the relationship was more likely due to the small mass range studied rather than to any fundamental differences between size and respiration for *M. relicta* compared with observed respiration-size relationships over a wide size range. The decrease in respiration with increasing size may reflect changes in the amount of respiring tissue compared to the total organism. For many invertebrates, increases in size are accompanied by increases in the relative lipid content of the organisms as has been observed for *Diporeia* (Landrum and Stubblefield 1991). This often accompanies preparation for reproduction. Thus, the ratio of respiring tissue to total mass declines and results in reduced mass specific respiration.

Xenobiotic and Oxygen Clearances

Attempting to relate xenobiotic clearance to a basic metabolic parameter such as respiration is not new. The uptake of xenobiotics from water has been expressed mathematically as a function of ventilation or respiration rate for fish (Neely 1979, Spacie and Hamelink 1982). Further, the quantitative relationships and mechanisms for the uptake of oxygen and nonpolar organic xenobiotics by fish have been well established (Murphy and Murphy 1971; McKim and Goeden 1982; McKim and

Heath 1983; McKim *et al.* 1985, 1987a, 1987b). However, direct experimental evidence supporting such mathematical expressions is minimal for invertebrates, and simultaneous measures of respiration and xenobiotic accumulation have only recently been made for the amphipod, *Diporeia* (Landrum and Stubblefield 1991). The rate of uptake of a chemical is considered to be first order with respect to the concentration of chemical in the water and can theoretically be related to animal respiration in the following equation:

$$dC_a/dt = k_w C_w = ERC_w/W \quad (4)$$

where

- C_a = concentration (ng g^{-1}) of xenobiotic in the animal,
- k_w = uptake clearance of xenobiotic from water ($\text{mL g}^{-1} \text{h}^{-1}$),
- C_w = concentration (ng mL^{-1}) of xenobiotic in water,
- E = transfer efficiency of the xenobiotic,
- R = respiratory ventilation volume (mL h^{-1}), and
- W = mass (g) of the animal.

Theoretically, the ratio of xenobiotic clearance to oxygen clearance is equal to the oxygen-specific xenobiotic transfer efficiency (Norstrom *et al.* 1976). However, other factors may affect the observed k_w .

Factors such as changes in organism physiology throughout the year, aqueous solubility of the xenobiotic, and variation in bioavailability can all impact the estimate of xenobiotic clearance from water for *Mysis relicta* (Frez and Landrum 1986). In fact, binding of chemicals to dissolved organic matter (DOM) reduces the available xenobiotic pool, thus decreasing the estimate of xenobiotic clearance when calculated on the total xenobiotic water concentration (Landrum *et al.* 1985, 1987). In the case of HCBP and BaP, the clearance of the xenobiotic would have been underestimated by as much as 30% if the appropriate corrections for binding to DOM had not been made.

For these studies, oxygen, BaP, and HCBP accumulation rate as represented by the uptake clearances were not strongly linked when all of the data were considered, in spite of the similar slopes for their regressions with mass. The large variability in the data was suggested as one possible reason for the absence of correlation. An unexplained problem in one experiment might also have contributed

to the failure to produce a significant relationship. However, other factors such as different dominant routes for the uptake of xenobiotics versus oxygen may also account for some of the variability. Since the ratios of xenobiotic to oxygen clearances are greater than one, either the compounds are accumulated more efficiently than oxygen or an additional route of uptake is available for the xenobiotics and not oxygen. In the amphipod, *Diporeia*, the accumulation of the organic xenobiotics was attributed in large part to transfer across the integument (Landrum and Stubblefield 1991). If a similar route dominated xenobiotic accumulation in mysids and if the respiratory membrane is small relative to the overall integument, then there would be increased variability and less correspondence between oxygen clearance and xenobiotic clearance. It will require more study to determine which mechanism is responsible for the observed results.

The clearances for the two xenobiotics were very strongly correlated. The slope of the regression line between the two is less than one and suggests that BaP was accumulated less efficiently than HCBP. Thus, BaP likely encounters more resistance than HCBP for transfer across the membranes.

SUMMARY

The two xenobiotics did not affect mysid respiration at the levels of exposure, and their accumulation was not as tightly coupled to respiration as expected. This may occur in part because the xenobiotics are taken up by different routes than oxygen. Xenobiotic accumulation was not completely uncoupled from respiration, based on the regression relationships between clearance and mass, the generation of significant regressions between oxygen clearance and xenobiotic clearance if the last experiment is omitted, and the ratios of xenobiotic clearances to oxygen clearance. Further study will be required to more specifically define the relationship between organism size, respiration, and xenobiotic accumulation for *Mysis relicta*.

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